VERIFICATION OF TRANSLATION

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declare as follows:

1. That I am well acquainted with both the English and Japanese languages, and

2. That the attached document is a true and correct translation made by me to the best of my knowledge and belief of:-

(a) US Application No. 10/611,310
Entitled: "METHODS OF TESTING FOR ALLERGIC DISEASES AND THERAPEUTIC AGENTS FOR TREATING SAME"
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METHODS OF TESTING FOR ALLERGIC DISEASES AND THERAPEUTIC AGENTS FOR TREATING SAME

FIELD OF THE INVENTION

The present invention relates to methods of testing for allergic diseases, and methods of screening candidate compounds for therapeutic agents for allergic diseases, using the expression of the allergic disease-related TR3 or "TINUR genes as an index. The present invention is also directed to pharmaceutical agents for treating allergic diseases.

BACKGROUND OF THE INVENTION

Allergic diseases such as atopic dermatitis are considered to be multifactorial diseases. Multifactorial diseases are caused by the interaction of many different genes, the expression of each of which is independently influenced by multiple environmental factors. Thus, determining the specific genes that cause a specific allergic disease is extremely difficult.

Allergic diseases are generally presumed to be associated with the expression of genes having mutations or defects, and/or with the overexpression or reduced expression of specific genes. To determine the role of gene expression in a disease, it is necessary to understand how genes are involved in the onset of that disease, and how gene expression is altered by external stimulants such as drugs.

Recent developments in gene expression analysis techniques have enabled analysis and comparison of gene expression in multiple clinical samples. As an example of such methods, the differential display (DD) method is useful. Liang and Pardee originally developed this method in 1992 (Science, 1992, 257: 967-971). By using this method, several dozen or more samples can be screened at one time, thereby enabling the detection of genes whose expression in one sample differs from other samples. Information essential to determining the causative gene(s) of a disease is expected to be obtained by examining genes with mutations, or genes whose expression changes depending on time and the environment, including genes whose expression is influenced by environmental factors.

Recently, patient interviews and patient medical and family history have become important factors in the diagnosis of allergic disease. More objective methods of diagnosing allergies include testing patient blood samples and observing patient immune response to allergen(s). Examples of the former method include allergen-specific IgE measurement, the leukocyte histamine release test and the lymphocyte blast transformation test. The presence of allergen-specific IgE is evidence of an allergic reaction against an allergen. However, allergen-specific IgE is not always detected in every patient. Furthermore, in principle, IgE assaying requires tests to be performed on all of the allergens necessary for diagnosis. The leukocyte histamine release test and the lymphocyte blast transformation test are methods for observing immune system reaction towards a specific allergen *in vitro*. Operation of these methods is complex.

Another known method useful in allergy diagnosis is based on the immune response observed when a patient contacts an allergen (i.e., the latter method). Such tests include the prick test, scratch test, patch test, intradermal reaction and induction test. These tests allow direct diagnosis of a patient's allergic reaction, but are highly invasive as patients are actually exposed to allergens.

Methods of confirming the involvement of an allergic reaction, regardless of allergen type, are also being trialed. For example, a high serum IgE titer indicates an allergic reaction in a patient. The serum IgE titer corresponds to the total amount of allergen-specific IgE. Determining the total amount of IgE is simple, regardless of the type of allergen; however, IgE titer may be reduced in some patients, for example, in those with non-atopic bronchitis.

The number of eosinophils and the level of eosinophil cationic protein (ECP) are diagnostic items for delayed-type reactions following Type I allergy and allergic inflammatory reactions. The number of eosinophils is considered to reflect the progress of allergic symptoms. ECP, a protein contained in eosinophil granules, is also strongly activated in patients having an asthma attack. Although allergic symptoms can indeed be identified using these diagnostic items, the extent to which they can actually be used as

diagnostic indices is limited.

Therefore, diagnostic indices useful in understanding pathological conditions in patients with allergic diseases, and in determining treatment regimens for such diseases, regardless of the type of allergen, have been greatly sought after. Allergic disease markers that are less risky for patients and capable of readily providing information required for diagnosis would be of great use. If genes associated with allergic disease can be identified, the expression of such genes can be used as an index to test for allergic diseases. Furthermore, if the cellular function of proteins encoded by these genes can be elucidated, observations regarding these functions can be used as a base to promote the development of therapeutic methods and pharmaceutical agents for treating allergic diseases.

SUMMARY OF THE INVENTION

The present invention was achieved in light of the above context. An objective of the present invention is to identify genes associated with allergic diseases. Furthermore, using expression of these genes as an index, another objective of the present invention is to provide methods of testing for allergic diseases, and methods of screening candidate compounds for therapeutic agents for allergic diseases, as well as pharmaceutical agents for treating allergic diseases.

The present inventors performed extensive analyses to achieve the above-mentioned objectives. Eosinophils commonly serve as typical clinical indicators of atopic dermatitis. Thus, the present inventors considered that if a gene whose expression level changes with eosinophil levels could be isolated, it could lead to the isolation of a gene directly involved in atopic dermatitis.

The present inventors first attempted to identify a gene whose expression level differs with a specific allergic disease. Differential expression comparative analysis using a gene chip was carried out on genes expressed in the peripheral blood eosinophils of healthy subjects, and of atopic dermatitis patients with various pathological conditions (light, severe and steroid sensitive, and severe and steroid resistant). Genes showing a greater than 3-fold

variation were sorted, and the TR3 gene was selected from among approximately 12,000 A-chip genes, wherein the chip was mainly loaded with known genes. Two cases of eosinophil RNA from each group, including the healthy subjects, were applied to the gene chip, and expression comparison between two groups was carried out by comparing gene expression in four combinations of two cases from each group. Comparison of expression between healthy subjects and subjects with severe symptoms (steroid sensitive) showed that TR3 expression varied by more than three-fold (enhanced in severe symptoms) in all four combinations. To confirm those observations, RT-PCR was carried out on panels of peripheral blood eosinophils having a larger number of patients from healthy subjects and atopic dermatitis patients. These results showed that TR3 expression in atopic dermatitis patients was enhanced as compared to that in healthy subjects, thus reproducing the results obtained using the gene chip.

TR3 is known as an α-type of the nuclear orphan receptor subfamily; however, to date it has not been reported as being related to allergic disease.

TINUR is a β-type of the nuclear orphan receptor subfamily, and predicted to be functionally similar to TR3. In the same manner as for TR3, the present inventors carried out a comparison of TINUR expression between healthy subjects and patients, using ABI7700 and the same panel of patient peripheral blood eosinophils, in which there were more than ten samples per group. The results confirmed that, regardless of symptom severity, TINUR gene expression was significantly enhanced in atopic dermatitis patients as compared to healthy subjects. Like the TR3 gene, a relationship between the TINUR gene and allergic disease has not yet been reported.

Genes suggestive of apoptotic character are found in the peripheral blood eosinophils of atopic dermatitis patients. This may be because negative feedback regulation acts to reduce the increase in peripheral blood eosinophils that occurs in association with a pathologic condition.

Allergic diseases may be tested by using the expression level of the TR3 or TINUR gene of this invention as an index.

The TR3 and TINUR receptors are orphan receptors, and hitherto,

neither their native ligands nor activators have been found. The present inventors developed a high-throughput system for searching for ligands, and using this system, succeeded in obtaining compounds that may function as activators of TR3 or TINUR transcription. These compounds are prostaglandins (PGA derivatives) comprising a cyclopentenone structure, and may be native ligands of the TR3 or TINUR receptor. Experiments using mutants in which a receptor's ligand-binding domain (LBD) region had been deleted indicated that the prostaglandin derivatives function by acting on this region. Moreover, experiments utilizing BIAcor demonstrated that PGA derivatives bind to TR3 and TINUR.

Thus, the present inventors found that it is possible to screen candidate compounds for a therapeutic agent for an allergic disease, and that PGA derivatives are TR3 or TINUR ligand activators.

The present inventors used a pharmacophore model to simulate the binding site of a PGA derivative TR3 ligand binding domain. The present inventors selected compounds from the database based on structure-activity relationship information for the PGA derivative reporter system. The present inventors selected compounds other than PGA derivatives that matched the binding pocket. These compounds are expected to function as TR3 receptor ligands.

Compounds that induce TR3 or TINUR gene expression, or compounds that bind to the TR3 or TINUR receptor and promote transcription activity (for example, ligand activators) are expected to have therapeutic effects on allergic diseases.

Furthermore, the present inventors discovered for the first time that the expression of TR3 and TINUR in cultured peripheral blood eosinophils is dramatically induced by apoptosis stimulation of cells via an anti-CD30 antibody comprising agonist activity towards eosinophil CD30. Therefore, a therapeutic agent for allergic diseases can be provided, wherein such an agent increases TR3 or TINUR gene expression using eosinophil CD30 ligand stimulation, and induces eosinophil apoptosis by regulating the expression of genes downstream of TR3 or TINUR occurring in eosinophils.

The present invention relates to a method of testing for allergic diseases, and a method of screening candidate compounds for

therapeutic agents for allergic diseases. These methods are performed using, as an index, expression of the TR3 or TINUR gene, which are genes highly expressed in activated eosinophils during allergic disease. The present invention also relates to pharmaceutical agents for treating allergic diseases. Specifically, the present invention provides:

[1] a method of testing for an allergic disease, said method comprising the steps of:

a) measuring the expression level of a TR3 or TINUR receptor protein, or a gene encoding the TR3 or TINUR receptor protein, in eosinophil cells of a test subject; and

b) comparing the expression level of the protein or gene in the eosinophil cells of the test subject with an expression level in eosinophil cells of a healthy subject.

[2] the testing method of claim 1, wherein the gene expression level is measured by cDNA PCR.

[3] the testing method of claim 1 or 2, wherein the allergic disease is atopic dermatitis.

[4] a reagent for testing for an allergic disease, said reagent comprising an oligonucleotide of at least 15 nucleotides in length that comprises a nucleotide sequence complementary to a polynucleotide encoding a TR3 or TINUR receptor protein, or to its complementary strand.

[5] a method of detecting the influence of a candidate compound on the expression level of a polynucleotide of (a) or (b) below, wherein said method comprises the steps of:

(1) contacting the candidate compound with a cell that expresses a polynucleotide of (a) or (b):

(a) a polynucleotide encoding a TR3 or TINUR receptor protein; and

(b) a polynucleotide encoding a protein whose expression in the eosinophils of an atopic dermatitis patient is increased, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide encoding a TR3 or TINUR receptor protein; and

(2) measuring the expression level of the polynucleotide of (a) or (b).

[6] the method of claim 5, wherein the cell is from a leukocyte cell line.

[7] a method of detecting the influence of a candidate compound on the expression level of a polynucleotide of (a) or (b) below, wherein said method comprises the steps of:

(1) administering the candidate compound to a test animal; and

(2) measuring the expression intensity of a polynucleotide in the eosinophil cells of the test animal, wherein the polynucleotide is selected from (a) or (b):

(a) a polynucleotide encoding a TR3 or TINUR receptor protein; and

(b) a polynucleotide encoding a protein whose expression in the eosinophils of an atopic dermatitis patient is increased, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide encoding a TR3 or TINUR receptor protein.

[8] a method of screening for a compound that increases the expression level of the polynucleotide (a) or (b), wherein said method comprises the steps of detecting the influence on expression level by the method of any one of claims 5 to 7, and selecting a compound that increases that expression level as compared to a control.

[9] a method of detecting the influence of a candidate compound on the expression level of a polynucleotide encoding a TR3 or TINUR receptor protein, wherein said method comprises the steps of:

(1) contacting a candidate compound with a cell or cell extract containing a DNA comprising a structure such that a reporter gene and the transcription regulatory region of a gene encoding a TR3 or TINUR receptor protein are operably linked; and

(2) measuring the activity of the reporter gene.

[10] a method of screening for a candidate compound that increases the expression level of a gene encoding a TR3 or TINUR receptor protein, wherein said method comprises the steps of detecting the influence of a compound on the activity of the reporter gene by the method of claim 9, and selecting a compound that increases the activity compared to a control.

[11] a method of screening candidate compounds for a therapeutic agent for an allergic disease, wherein said method comprises the steps

of:

1) contacting a test compound with a TR3 or TINUR receptor protein;

2) measuring the binding activity between the test compound and the TR3 or TINUR receptor protein; and

3) selecting the compound that binds to the TR3 or TINUR receptor protein.

[12] a method of screening candidate compounds for a therapeutic agent for an allergic disease, wherein said method comprises the steps of:

1) providing cells transfected with (a) a DNA that can express a fusion protein of a TR3 or TINUR receptor protein or its ligand binding domain and a transcription regulatory region binding protein, and (b) a DNA having a reporter gene is operably linked downstream of a DNA sequence to which the transcription regulatory region binding protein binds;

2) contacting the cell with the test compound;

3) measuring the activity of the reporter gene; and

4) selecting the compound that changes this activity.

[13] a therapeutic agent for an allergic disease, said agent comprising, as an active ingredient, a compound obtainable by the screening method of any one of claims 10 to 12.

[14] a therapeutic agent for an allergic disease, said agent comprising, as an active ingredient, a prostaglandin comprising a cyclopentenone structure and that is obtainable by the screening method of any one of claims 10 to 12.

[15] a therapeutic agent for an allergic disease, said agent comprising, as an active ingredient, a ligand of a TR3 or TINUR receptor.

[16] the therapeutic agent for an allergic disease of claim 15, wherein the ligand of a TR3 or TINUR receptor is a prostaglandin comprising a cyclopentenone structure.

[17] the therapeutic agent for an allergic disease of claim 16, wherein the prostaglandin having a cyclopentenone structure is selected from the group consisting of prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2,

16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin A_1, 15-deoxy-delta 12,14-prostaglandin J_2, and 8-isoprostaglandin A_1.

[18] the therapeutic agent for an allergic disease of claim 15, wherein the ligand of a TR3 receptor is any one of the compounds listed in Tables 14 to 49.

[19] the therapeutic agent for an allergic disease of any one of claims 13 to 18, wherein the allergic disease is atopic dermatitis.

[20] an animal model for an allergic disease, wherein the animal is a transgenic non-human vertebrate in which the expression intensity of polynucleotide (a) or (b) below is decreased in eosinophil cells:

(a) a polynucleotide encoding a TR3 or TINUR receptor protein; and

(b) a polynucleotide encoding a protein whose expression in the eosinophils of an atopic dermatitis patient is increased, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide encoding a TR3 or TINUR receptor protein.

[21] the animal model of claim 20, wherein the transgenic animal is a knockout animal.

[22] a method of inducing cell apoptosis, said method comprising activation of a TR3 or TINUR receptor protein in the cell.

[23] the apoptosis induction method of claim 22, which comprises the step of contacting a cell with a compound that is obtainable by the screening method of any one of claims 10 to 12, or a prostaglandin comprising a cyclopentenone structure.

[24] the apoptosis induction method of claim 22 or 23, wherein said cell is an eosinophil cell.

[25] an apoptosis-inducing agent, which comprises a compound or a prostaglandin comprising a cyclopentenone structure and that is obtainable by the screening method of any one of claims 10 to 12.

[26] an apoptosis-inducing agent comprising a ligand of a TR3 or TINUR receptor as an active ingredient.

[27] the apoptosis-inducing agent of claim 26, wherein the ligand of the TR3 or TINUR receptor is a prostaglandin comprising a cyclopentenone structure.

[28] the apoptosis-inducing agent of claim 27, wherein the

prostaglandin comprising a cyclopentenone structure is selected from the group consisting of prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin A_1, 15-deoxy-delta 12,14-prostaglandin J_2, and 8-isoprostaglandin A_1.

[29] the apoptosis-inducing agent of claim 26, wherein the ligand of the TR3 receptor is any one of the compounds listed in Tables 14 to 49.

[30] a TR3 or TINUR gene expression-inducing agent, which comprises a ligand of an eosinophil CD30 receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a graph of Table 6.

Fig. 2 shows a schematic illustration of a ligand searching system for a TR3 or TINUR receptor constructed by the present inventors. A TR3 or TINUR ligand-binding site is inserted into X, and the full-length retinoic acid X receptor (RXR)α gene is inserted into Y. These constructs are transfected into NIH3T3 cells, and the activity of induced luciferase is measured.

Fig. 3 shows a schematic illustration of the structure of the TR3 and TINUR receptor proteins.

Fig. 4 shows a graph demonstrating the transcription-activating function of TR3 in a series of cyclopentenone prostaglandins using the system of Fig. 2.

Fig. 5 shows a graph demonstrating the results of measuring the expression levels of the TINUR gene in healthy subjects and patients using ABI7700.

Fig. 6 shows a graph demonstrating the transcription-activating function of the TINUR gene in a series of cyclopentenone prostaglandins using the system of Fig. 2.

Fig. 7 shows diagram of prostaglandin A_2 in alpha model, in which the binding position of the PGA derivative for the TR3 ligand binding domain has been simulated using the Pharmacophore model.

Fig. 8 shows a graph demonstrating the decrease of prostaglandin

A_2 transcriptional activity by the LBD deletion mutant. ΔLBD denotes the deletion mutant.

Fig. 9 shows diagrams showing PGA1 and PGA2 bound to TR3 LBD or TINUR LBD, revealed using BIAcor S51. Glutathione S-transferase (GST) was used as a comparison control, and 13,14-Dihydro-15-keto-PGA2 was used as a negative control.

Fig. 10 shows graphs demonstrating the results of TR3 expression induction in apoptotic stimulation of peripheral blood eosinophils using an anti-CD30 or anti-Fas antibody. Beta-actin-corrected values and GAPDH-corrected values are shown.

Fig. 11 shows graphs demonstrating the results of TINUR expression induction in apoptotic stimulation of peripheral blood eosinophils using anti-CD30 or anti-Fas antibody. Beta-actin-corrected values and GAPDH-corrected values are shown.

Fig. 12 is a graph indicating apoptosis induction after treating the eosinophil-specific cell line, AML14.3D10, with anti-CD30 antibody or anti-Fas antibody.

Fig. 13 is a graph indicating TR3 expression induction after treating the eosinophil-specific cell line, AML14.3D10, with anti-CD30 antibody or anti-Fas antibody.

Fig. 14 shows a graph indicating TINUR expression induction after treating the eosinophil-specific cell line, AML14.3D10, with anti-CD30 antibody or anti-Fas antibody.

Fig. 15 shows a working hypothesis for allergic disease treatment via eosinophil cell death, caused by a member of the nuclear receptor Nur subfamily, including TR3 and TINUR.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors discovered that the expression level of the TR3 and/or TINUR genes increases in the eosinophils of atopic dermatitis patients. Therefore, using TR3 and/or TINUR gene expression level as an index, tests for allergic disease can be performed on test subjects.

The present invention provides methods for testing for an allergic disease, which comprise the step of measuring the expression level of the TR3 or TINUR gene.

A preferred embodiment of the present invention includes the following steps:

(a) measuring the expression level of a gene encoding the TR3 or TINUR receptor protein in the eosinophil cells of a test subject; and

(b) comparing this measured value to that measured in the eosinophils of a healthy subject.

The TR3 and TINUR receptors are α and β-type orphan nuclear receptors respectively, wherein orphan nuclear receptors are composed of three subfamilies. As shown in Table 1, orphan nuclear receptors have various names, and the terms "TR3 gene" and "TINUR gene" as used in the context of the present invention should not necessarily be construed as being limited to human-derived genes.

Table 1

	Human	Mouse	Rat
α	NAK-1 (TR3)	nur77	NGFI-B
β	TINUR/NOT	Nurr1	RNR-1
γ	MINOR/CHN	TEC	NOR-1

Information relating to the amino acid sequences of these TR3 and TINUR receptor proteins, and the nucleotide sequences of genes encoding these proteins, can be readily obtained from various gene databases available to those skilled in the art. Specifically, the nucleotide sequence of a gene encoding the human TR3 receptor protein (TR3 gene) is shown in SEQ ID NO: 1; and the amino acid sequence of the human TR3 receptor protein is shown in SEQ ID NO: 2. The nucleotide sequence of a gene encoding the human TINUR receptor protein (TINUR gene) is shown in SEQ ID NO: 3; and the amino acid sequence of the human TINUR receptor protein is shown in SEQ ID NO: 4.

Herein, the general phrase "allergic disease" refers to a disease involving allergic reactions. More specifically, an "allergic disease" is defined as a disease for which an allergen is identified, where there is a strong correlation between exposure to that allergen and the onset of pathological change, and where that

pathological change has been proven to have an immunological mechanism. Herein, an immunological mechanism means that leukocytes show an immune response to allergen stimulation. Examples of allergens include mite antigens and pollen antigens.

Representative allergic diseases include bronchial asthma, allergic rhinitis, atopic dermatitis, and pollen and insect allergies. Allergic diathesis is a genetic factor that can be inherited by the children of allergic parents. Familial allergic diseases are also called atopic diseases, and the causative, genetically transmitted factor is atopic diathesis. "Atopic dermatitis" is a general term for an atopic disease, especially diseases accompanied by dermatitis symptoms.

The tests for allergic diseases of the present invention can include, for example, a test for determining whether a subject is affected with an allergic disease, a test for determining whether a subject comprises the trait of being easily affected by an allergic disease, and a test for assessing whether allergic symptoms are improving. The TR3 or TINUR gene of this invention showed increased expression level in the activated eosinophils of atopic dermatitis patients. Since eosinophils are a representative clinical marker for atopic dermatitis, a clinical marker associated with their decrease is useful for assessing therapeutic effects. More specifically, increased TR3 or TINUR gene expression indicates improvement of the allergic disease, accompanied by a decrease in eosinophils.

There is a correlation between atopic dermatitis severity and the number of eosinophils, such that active reduction of eosinophil number may lead to curing the disease. Measurement of these genes, whose specific induction in eosinophils is accompanied by a decrease in eosinophil numbers, along with discovery of methods or substances that actively induce these genes from outside the cell, may lead to novel methods of atopic dermatitis therapy, and diagnostic methods for evaluating these therapeutic methods.

Herein, the expression level of the TR3 or TINUR gene includes transcription of the gene to mRNA, as well as translation into their protein. Therefore, a method of testing for an allergic disease according to the present invention can be performed by comparing the

expression intensity of mRNA corresponding to the particular gene, or the expression level of the protein encoded by that gene.

Measurement of TR3 or TINUR gene expression level in a method of testing for allergic diseases of the present invention may be conducted according to gene analytical methods known to those skilled in the art. More specifically, a hybridization technique using as a probe a nucleic acid that hybridizes to either the TR3 or TINUR gene, or a gene amplification technique using as a primer a DNA that hybridizes to a gene of this invention, or such can be utilized.

Primers or probes that can be used as reagents for testing for an allergic disease according to the present invention include a polynucleotide comprising at least 15 nucleotides that is complementary to the nucleotide sequence of SEQ ID NO: 1 or 3, or the complementary strand thereof. Herein, the term "complementary strand" refers to the other strand of one strand of a double stranded DNA, which is composed of A:T (or A:U for RNA) and G:C base pairs. In addition, "complementary" means not only those sequences completely complementary to a region of at least 15 continuous nucleotides, but also those having a homology of at least 70%, preferably at least 80%, more preferably 90%, and even more preferably 95% or higher. The degree of homology between nucleotide sequences can be determined using a known algorithm, such as BLASTN.

Such polynucleotides are useful as probes to detect and isolate a polynucleotide encoding a protein of the present invention, or as primers to amplify a polynucleotide of the present invention. When used as a primer, these polynucleotides have a chain length of usually 15 bp to 100 bp, and preferably 15 bp to 35 bp. When used as a probe, DNAs comprising the entire sequence of a polynucleotide of the present invention, or its partial sequence containing at least 15-bp, are used. When used as a primer, the 3′ region must be complementary to a polynucleotide of the present invention, however the 5′ region can be linked to a restriction enzyme-recognition sequence, tag, or the like.

A "polynucleotide" of the present invention may be either DNA or RNA. These polynucleotides may be either synthetic (isolated) or naturally occurring. In addition, DNA used as a hybridization probe

is preferably labeled. Examples of labeling methods are described below. Herein, the term "oligonucleotide" refers to polynucleotides with a relatively low degree of polymerization. Oligonucleotides are included in polynucleotides. Exemplary labeling methods are as follows:

·nick translation labeling using DNA polymerase I;

·end labeling using polynucleotide kinase;

·fill-in end labeling using the Klenow fragment (Berger, SL, Kimmel, AR. (1987) Guide to Molecular Cloning Techniques, Method in Enzymology, Academic Press; Hames, BD, Higgins, SJ (1985) Genes Probes: A Practical Approach. IRL Press; Sambrook, J, Fritsch, EF, Maniatis, T. (1989) Molecular Cloning: a Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press);

·transcription labeling using RNA polymerase (Melton, DA, Krieg, PA, Rebagkiati, MR, Maniatis, T, Zinn, K, Green, MR. (1984) Nucleic Acid Res., 12, 7035-7056); and

·non-radioisotopic labeling of DNA by incorporating modified nucleotides (Kricka, LJ. (1992) Nonisotopic DNA Probing Techniques. Academic Press).

When testing for allergic diseases using hybridization techniques, for example, Northern hybridization, dot blot hybridization or DNA microarray techniques may be used. Gene amplification techniques such as RT-PCR may also be used. During the gene amplification step of RT-PCR, PCR amplification monitoring can be used to quantitatively analyze expression of the gene of the present invention.

In PCR gene amplification monitoring, the detection target (the DNA or reverse transcript of RNA) is hybridized to probes that are dual-labeled at both ends with different fluorescent dyes, whose fluorescence cancels each other out. As the PCR proceeds and the Taq polymerase degrades the probe due to its 5'-3' exonuclease activity, the two fluorescent dyes become distant from each other and fluorescence is detected. Fluorescence is detected in real time. By simultaneously measuring a standard sample in which the target copy number is known, it is possible to use cycle number to determine the target copy number of the subject sample, when PCR amplification is

linear (Holland, P. M. *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88: 7276-7280; Livak, K. J. *et al.*, 1995, PCR Methods and Applications 4(6): 357-362; Heid, C. A. *et al.*, 1996, Genome Research 6: 986-994; Gibson, E. M. U. *et al.*, 1996, Genome Research 6: 995-1001). For example, ABI PRISM7700 (PE Biosystems) may be used for the PCR amplification monitoring method.

A method of testing for allergic diseases of the present invention can also be carried out by detecting a protein encoded by the TR3 or TINUR gene. Test methods that may be employed include those using an antibody that binds to a protein encoded by the TR3 or TINUR gene, such as Western blotting, immunoprecipitation and ELISA.

Antibodies that bind to the TR3 or TINUR protein used in the detection step may be produced by techniques well known to those skilled in the art. Antibodies used in the present invention may be polyclonal or monoclonal (Milstein, C. *et al.*, 1983, Nature 305 (5934): 537-40). For example, polyclonal antibodies against a protein of the present invention may be produced by collecting blood from mammals sensitized with an antigen, and separating serum from this blood using known methods. Serum containing polyclonal antibodies may be used as polyclonal antibodies. A fraction containing polyclonal antibodies can be further isolated from this serum as required. Alternatively, monoclonal antibodies may be obtained by isolating immune cells from mammals sensitized with an antigen, fusing these cells with myeloma cells or the like, cloning the hybridomas thus obtained, and collecting the antibodies from the culture for use as monoclonal antibodies.

These antibodies may be appropriately labeled to detect the TR3 or TINUR protein. Alternatively, instead of labeling these antibodies, a substance that specifically binds to these antibodies, for example, protein A or protein G, may be labeled to indirectly detect the protein. ELISA is one example of such an indirect detection method.

A protein or its partial peptide to be used as an antigen may be obtained by: 1) inserting the TR3 or TINUR gene, or a portion of the TR3 or TINUR gene, into an expression vector, 2) introducing the vector into an appropriate host cell to produce a transformant, 3)

culturing the transformant to express the recombinant protein, and 4) purifying the expressed recombinant protein from the culture or the culture supernatant. Alternatively, oligonucleotides consisting of a partial amino acid sequence of the amino acid encoded by the TR3 or TINUR gene can be chemically synthesized and used as the immunogen.

The samples of this invention are preferably eosinophils derived from test subjects. Eosinophils can be prepared from peripheral blood using conventional methods. For example, leukocytes are isolated by fractionating heparinized blood using centrifugation. Granulocytes can then be fractionated by, for example, Ficoll centrifugation of the leukocytes. Eosinophils can be then isolated by neutrophil depletion using the CD16 antibody. A sample for immunological assays of the aforementioned proteins can then be obtained by disrupting these isolated eosinophils to produce a lysate. Alternatively, a sample for measuring mRNA corresponding to the aforementioned gene can be obtained by extracting mRNA from this lysate. The use of a commercially available kit is useful in extracting mRNA or preparing eosinophil lysate.

In the present invention, the expression level of the gene serving as the index can be measured from whole blood or a peripheral blood leukocyte population, without isolating eosinophils. In this case, the change of gene expression level in cells can be determined by correcting measured values. For example, the measured expression level of an index gene of the present invention can be corrected based on the measured expression level of a housekeeping gene, that is, a gene specifically expressed in eosinophils, and whose expression level does not significantly change, regardless of cellular conditions.

Alternatively, where the protein to be detected is a secretory protein, comparison of the expression level of a gene encoding the protein can be accomplished by measuring the amount of the target protein in a sample of the subject's body fluid, such as blood or serum.

When the result of a test for allergic disease of this invention shows elevated expression of a gene of this invention, allergic

symptoms are presumed to be improving together with a decrease in eosinophils. This is especially the case for patients with an allergic disease such as atopic dermatitis

Furthermore, this invention also relates to an allergic disease animal model, wherein said animal is a transgenic non-human animal having decreased expression of the polynucleotide of (a) or (b) in eosinophil cells:

(a) a polynucleotide encoding the TR3 or TINUR receptor protein; and

(b) a polynucleotide encoding a protein whose expression in the eosinophils of atopic dermatitis patients is increased, wherein the polynucleotide hybridizes under stringent conditions with a polynucleotide encoding the TR3 or TINUR receptor protein.

According to this invention, a decrease in expression level includes a knockout condition in which gene function has been substantially repressed. Herein, substantial repression of gene function refers to a condition in which neither expression of the gene, nor activity of the protein encoded by that gene, can be observed. Gene expression level can be confirmed by quantitative PCR, such as that shown in the Examples. Comparison with normal conditions can be used to confirm that translation product protein activity is virtually undetectable.

Such transgenic animals include animals that are incapable of expressing the original protein activity due to, for example, the introduction of a mutation into the coding region of the gene, which artificially causes an amino acid sequence mutation, or the introduction of a stop codon. Examples of amino acid sequence mutations include substitution, deletion, insertion and addition of amino acid(s). In addition, by mutating the transcriptional regulatory region of the gene, the actual expression of the gene of this invention can be controlled.

Methods for obtaining transgenic animals comprising a particular target gene are known. For example, a transgenic animal can be obtained by a method wherein a gene and an ovum are mixed and treated with calcium phosphate; a method wherein the gene is directly introduced into the nucleus of an pronuclear-stage oocyte using a

micropipette under a phase contrast microscope (microinjection method, US Patent No. 4873191); a method wherein embryonic stem cells (ES cells) are used; etc. Other methods have also been developed, including a method for infecting ovum with a retroviral vector in which a gene has been inserted, and a method for transducing a gene into ovum via sperm. This latter sperm vector method is a gene recombination technique whereby an exogenous gene is introduced into an ovum by fertilization with a sperm, wherein that exogenous gene has been incorporated into the sperm by adhesion, electroporation, or the like (M. Lavitranoet, *et al.* Cell, 57, 717, 1989).

Transgenic animals of the present invention can be produced using any vertebrate except humans. Transgenic animals comprising various gene insertions and modified gene expression levels are currently being produced using vertebrates such as mice, rats, rabbits, miniature pigs, goats, sheep or cattle.

An example of a transgenic animal of this invention includes a knockout animal in which expression of a non-human homologue of the human TR3 or TINUR gene (described in SEQ ID NO: 1 and 3 respectively) is inhibited. Observation of the knockout animal phenotype enables knowledge of the specific function of the knocked out gene. The gene comprising the nucleotide sequence of SEQ ID NO: 1 or 3 showed increased expression in the eosinophils of human atopic dermatitis patients. Therefore, an animal in which a homologue of this gene is knocked out is useful as an animal model for allergic diseases.

For example, if a knockout animal of this invention develops dermatitis, or exhibits a change in measured values relating to some sort of allergic disease, a screening system can be constructed to search for a compound that comprises the function of facilitating recovery from that change.

Methods for producing knockout animals are well known. Using the example of a mouse, a known method for the production of a knockout animal is by homologous recombination using embryonic stem cells, and then selection of embryonic stem cells in which one of the alleles is modified or destroyed. A chimeric animal containing cells derived from an embryonic stem cell together with cells derived from an embryo

can be obtained, for example, by inserting a genetically manipulated embryonic stem cell into a fertilized egg. When this chimeric animal (chimera refers to a single individual formed from somatic cells derived from two or more fertilized eggs) is crossed with a normal mouse, a heterozygote in which one of the alleles is modified or destroyed in its entirety, can be produced. Furthermore, a homozygote can be produced by crossing heterozygotes. The transgenic animals of this invention include both heterozygotes and homozygotes.

Homologous recombination refers to a mechanism of genetic recombination that occurs between two genes comprising the same or very similar nucleotide sequences. PCR can be used to select cells that have undergone homologous recombination. A portion of an inserted gene, and a portion of the region in which insertion is expected, can be used as primers in a PCR reaction carried out to confirm homologous recombination in cells that produce amplification products. Furthermore, when inducing homologous recombination of a gene expressed in an embryonic stem cell, cell selection can be easily carried out using neomycin resistance, wherein a neomycin resistance gene has been linked to a transgene and introduced into a cell. This and other known methods, and modified methods thereof, can be used to select cells.

In addition to use in the screening of pharmaceutical agents for the treatment or prevention of allergic diseases, described below, transgenic animals of this invention are also useful for elucidating the mechanisms of allergic diseases, and for testing the safety of screened compounds.

The present invention revealed that expression of the TR3 and TINUR genes increases in the eosinophils of atopic dermatitis patients. This may be because negative feedback regulation acts to reduce the increase in peripheral blood eosinophils that occurs in association with a pathologic condition. Therefore, animals that can be used as allergic disease model animals include animals in which the expression level of the TR3, the TINUR gene, or a gene functionally equivalent to the TR3 or TINUR gene, has been artificially lowered in eosinophil cells. A decrease of gene expression level in eosinophils includes a decrease in the expression level over the entire leukocyte

population. In other words, this phrase includes decreased expression of the aforementioned genes not only in eosinophils but also over the general leukocyte population. In the present invention, a functionally equivalent gene normally refers to a gene of either (a) or (b), described above. More specifically, examples of functionally equivalent genes of this invention include genes that hybridize under stringent conditions to a gene that encodes TR3 or TINUR. Generally, the following conditions can be indicated as the stringent conditions of this invention. For example, hybridization in 4x SSC at 65°C followed by washing with 0.1x SSC at 65°C for one hour. The temperature conditions for hybridization and washing greatly influence stringency and can be adjusted using melting temperature (Tm). Tm varies with the ratio of constitutive nucleotides in the hybridizing base pairs, and with the composition of the hybridization solution (concentrations of salts, formamide and sodium dodecyl sulfate). Therefore, on considering these conditions, one skilled in the art can select appropriate conditions to achieve an equal stringency based experience or experimentation.

For example, the aforementioned transgenic animals may be used as the animal model of this invention.

Furthermore, the present invention provides a method for detecting the influence of a candidate compound on the expression level of a polynucleotide of this invention. According to this invention, TR3 or TINUR gene expression level is significantly increased in the eosinophils of atopic dermatitis patients. This is thought to be due to negative feedback regulation that acts to reduce the increase in peripheral blood eosinophils that occurs in association with a pathologic condition. Therefore, based on these methods for detecting influence on gene expression level, compounds that increase gene expression level can be selected, and therapeutic drugs for allergic diseases can be obtained. Herein, compounds that increase the expression level of a gene refer to compounds that comprise the function of inducing any one of the steps selected from gene transcription, translation and expression of protein activity. The present invention further provides a method for detecting the activity of the TR3 or TINUR gene product protein (transcriptional

activation ability), as well as TR3 or TINUR gene expression level. Therapeutic drugs for allergies can be devised by selecting compounds that increase the activity of the TR3 or TINUR gene product protein (transcriptional activation ability).

The method for detecting the influence of a candidate compound on the expression level of a polynucleotide of this invention can be performed *in vivo* or *in vitro*. To detect *in vivo* influence, an appropriate test animal should be used. Test animals that can be used include, for example, an allergic disease animal model, or an allergic disease animal model that is a transgenic non-human animal in which the expression of the aforementioned (a) or (b) gene is inhibited in eosinophils. *In vivo* influence on expression level based on the present invention can be detected, for example, according to the following steps:

(1) administering a candidate compound to a test animal; and

(2) measuring the expression level of the polynucleotide of the above-described (a) or (b) in the eosinophils of the test animal .

A test animal for the method of detection of this invention can also include, for example, transgenic animals in which TR3 or TINUR gene expression has been decreased through the expression of a TR3 or TINUR antisense gene. Such transgenic animals may be produced by first constructing an antisense RNA expression vector by inserting the full-length TR3 or TINUR gene, or partial sequence thereof, in the reverse direction and downstream of an appropriate promoter sequence. This expression vector is then introduced into the nucleus to express a TR3 or TINUR antisense gene. Thus, a transgenic animal with reduced TR3 or TINUR gene expression can be obtained. When the expression vector contains a promoter whose transcription is regulated by an appropriate pharmaceutical substance, TR3 or TINUR gene expression level in the transgenic animal can be controlled by administering that substance.

The influence of a pharmaceutical agent candidate compound on TR3 or TINUR gene expression can be detected by administering that compound to an animal model in which TR3 or TINUR gene expression has been reduced as described above, and then monitoring the effect of that compound on TR3 or TINUR gene expression in the eosinophils

of that animal model.

The method of screening of this invention allows selection of pharmaceutical agents involved in TR3 or TINUR gene expression in various ways. For example, this invention enables the discovery of pharmaceutical agent candidate compounds having any of the following functions:

- Activating a signal transduction pathway that drives TR3 or TINUR gene expression;
- Increasing TR3 or TINUR gene transcription activity;
- Inhibiting degradation or stabilization of the TR3 or TINUR gene transcription product; etc.

In vitro detection can be performed using a method wherein a candidate compound is contacted with cells expressing one of the above-described (a) or (b) genes, and the expression level of that gene is detected. More specifically, the method may be carried out according to the following steps:

(1) contacting a candidate compound with cells that express a polynucleotide of the above-described (a) or (b); and

(2) measuring the expression level of that polynucleotide of the above-described (a) or (b).

In this invention, cells to be used in step (1) can be obtained by inserting such a polynucleotide into an appropriate expression vector, and then transfecting suitable host cells with that vector. Vector or host cells capable of expressing a gene of this invention should be used. Examples of host cells in the host-vector system are *Escherichia coli*, yeast cells, insect cells, animal cells and the like. Vectors for use with each of these cells can be routinely selected.

Vectors may be transfected into a host by biological, physical or chemical methods. Biological methods include, for example, methods using viral vectors; methods using specific receptors; and cell-fusion methods (HVJ (hemagglutinating virus of Japan; Sendai virus) method, polyethylene glycol (PEG) method, electric cell fusion method, and microcell fusion method (chromosome transfer)). Examples of physical methods include microinjection, electroporation and the use of a gene particle gun. Chemical methods are exemplified

by the calcium phosphate precipitation method, liposome method, DEAE-dextran method, protoplast method, erythrocyte ghost method, erythrocyte membrane ghost method, and microcapsule method.

In a detection method of this invention, leukocyte cell lines can be used as cells for expressing the polynucleotide of the aforementioned (a) or (b). Examples of leukocyte cell lines are cell lines derived from leukocytes, such as Eol, YY-1, HL-60, TF-1 and AML14.3D10. Among the leukocyte cell lines, cell lines derived from eosinophils are preferred for a detection method of this invention. Examples of cell lines derived from eosinophils include Eol, YY-1 and AML14.3D10.

Eol (Eol-1: Saito H *et al.*, Establishment and characterization of a new human eosinophilic leukemia cell line. Blood 66, 1233-1240, 1985) can be obtained from the Hayashibara Research Institute. YY-1 (Ogata N *et al.*, The activation of the JAK2/STAT5 pathway is commonly involved in signaling through the human IL-5 receptor. Int. Arch. Allergy Immunol., Suppl 1, 24-27, 1997) is available from the Institute of Cytosignal Research. AML14.3D10 (Baumann MA *et al.*, The AML14 and AML14.3D10 cell lines: a long-overdue model for the study of eosinophils and more. Stem Cells, 16, 16-24, 1998) is commercially available from Paul CC at Research Service, VA Medical Center, Dayton, Ohio, USA.

HL-60 clone 15 (ATCC CRL-1964), an undifferentiated leukocyte cell line, will differentiate into eosinophils to produce an eosinophil cell line when cultured for about a week in the presence of butyric acid. Eosinophils are polymorphonuclear and exhibit eosinophilic granules, and can thus be detected by their morphological characteristics. Morphological observations are performed using Giemsa staining and Difquick staining. Generally, a human leukocyte cell line containing eosinophils can be established by cloning an immortalized cell sample from a leukemia patient. Therefore, one skilled in the art can use a conventional method to obtain an eosinophil cell line, as necessary. The method of screening involves the addition of a candidate compound to the aforementioned leukocyte cell line, measurement of the expression levels of the polynucleotides of (a) or (b) in the leukocyte cell line, and selection of a compound

that increases the gene expression level.

Transformed cells in which the expression of the polynucleotide of the aforementioned (a) or (b) is modified can be used as cells for the *in vitro* detection method. Examples of such transformed cells include cells transformed with an expression vector for the polynucleotide antisense. Cells transformed with an antisense expression vector can be obtained according to a principle similar to that used in the production of the aforementioned transgenic animal. Using the transformed cell thus obtained, the influence of the candidate compound on gene expression level can be detected.

In a method of the present invention, the expression levels of the polynucleotide of the above-described (a) or (b) can be compared by detecting the expression levels of not only proteins encoded by these genes, but also of their corresponding mRNAs. When comparing expression level using mRNA, the step of preparing an mRNA sample as described above is conducted instead of preparing a protein sample. Protein and mRNA detection can be carried out according to known methods, such as those described above.

By obtaining the transcriptional regulatory region of the TR3 or TINUR gene, a reporter assay system can be constructed. A reporter assay system is a system of screening for a transcriptional regulatory factor that acts on the transcriptional regulatory region. Such a system uses the expression level of a reporter gene located downstream of the transcriptional regulatory region, and expressed under the control of that regulatory region, as an index.

A transcriptional regulatory region is exemplified by a promoter and an enhancer, as well as a CAAT box, TATA box or the like, usually found in the promoter region. Examples of suitable reporter genes include the chloramphenicol acetyltransferase (CAT) gene, luciferase gene and growth hormone genes.

A transcriptional regulatory region of the TR3 or TINUR gene can be obtained using conventional methods as follows. First, a genomic DNA clone comprising the cDNA sequence based on a nucleotide sequence described in SEQ ID NO: 1 or 3, is screened by a method using PCR or hybridization from a human genomic DNA library, such as the BAC or YAC libraries. Based on the resulting genomic DNA sequence,

the transcriptional regulatory region of the TR3 or TINUR gene is predicted and obtained. A reporter construct is prepared by cloning the obtained transcriptional regulatory region upstream of a reporter gene. The resulting reporter construct is introduced into a cultured cell strain to prepare transformants for screening. By contacting a candidate compound with a transformant and detecting reporter gene expression, the effect of that candidate compound on the transcriptional regulatory region can be assessed.

Based on the methods for detecting influence on the expression level of the polynucleotides of the present invention, a compound that alters the expression level of these polynucleotides can be screened. The present invention relates to a method of screening for a compound that alters the expression level of a polynucleotide of above-described (a) or (b), comprising the steps below.

The present invention provides a method of screening for a compound that increases the expression level of a polynucleotide of above-described (a) or (b), the method comprising the steps of: 1) detecting the influence of a candidate compound on the expression level of the polynucleotide *in vivo* and/or *in vitro,* and 2) selecting the compound that increases expression level as compared to a control.

This invention also relates to a method of screening for a compound that acts on the transcriptional regulatory region, wherein that method uses a reporter assay which utilizes the transcriptional regulatory region of the TR3 or TINUR gene. A compound that increases reporter gene expression level as compared to a control can be selected based on the results of the reporter assay of the present invention, and a compound that induces TR3 or TINUR gene expression can thus be obtained. Thus, the present invention relates to a method of screening for agonists or antagonists that bind to the ligand-binding domain.

The TR3 and TINUR receptor proteins, discovered by the present inventors as proteins associated with allergic diseases, are orphan receptors and hitherto, their native ligand activators have not been found. TR3 or TINUR protein ligand activators are considered to directly activate TR3 or TINUR in eosinophils, and to promote apoptosis. Therefore, TR3 or TINUR receptor ligand activators are

expected to serve as therapeutic agents for allergic disease. Generally, a receptor ligand can be obtained by searching for compounds that bind to the receptor protein.

The present invention provides a method of screening candidate compounds for therapeutic agents for allergic disease, wherein such a method comprises selecting compounds that can bind to the TR3 or TINUR protein. In this method, the TR3 or TINUR receptor protein is contacted with a test compound, binding activity between each receptor protein and the test compound is measured, and a compound that binds to a receptor protein is selected. Agonists and antagonists can be selected by measuring this binding as well as by measuring TR3 or TINUR transcription activity.

The TR3 and TINUR receptor proteins of this method include their partial peptides. One skilled in the art can use known methods to measure binding activity between the TR3 or TINUR receptor protein and a test compound of the above-described method.

For example, if the compound that binds to TR3 or TINUR is a protein, West-Western blotting can be performed as the screening method of the present invention. Specifically, a cDNA library that uses a phage vector (λgt11, ZAPII, etc.) is constructed from tissues or cells predicted to express a protein (test protein) that binds to the TR3 or TINUR protein. This library is then expressed on LB-agarose, and expressed proteins are immobilized onto a filter. The TR3 or TINUR protein is purified as a biotin labeled protein, or as a fusion protein with the GST protein, and reacted with the above-mentioned filter. Binding activity can be evaluated by using streptavidin, anti-GST antibodies, or the like to detect plaques that express the test protein.

Another embodiment of the method for screening a candidate compound for an allergic disease therapeutic agent of this invention includes the steps of:

(1) providing cells transfected with (a) a DNA that can express a fusion protein of the TR3 or TINUR receptor protein or a ligand binding domain thereof, and a transcription regulatory region binding protein, and (b) a DNA comprising a reporter gene operably linked downstream of the DNA sequence to which the transcription regulatory region

binding protein binds;

(2) contacting the above-mentioned cells with a test compound;

(3) measuring the activity of the above-mentioned reporter gene; and

(4) selecting the compound that changes the above-mentioned activity.

The phrase "operably linked" in the above-mentioned method refers to a condition in which the reporter gene is bound such that it can be expressed when the TR3 or TINUR receptor protein, or the ligand binding domain of that protein, binds to a ligand of the receptor protein or to a ligand-like compound. The GAL4 protein can be preferably used as the "transcription regulatory region binding protein" in the above-mentioned method. Furthermore, the "DNA sequence to which a transcription regulatory region binding protein binds" can be, for example, a GAL4-binding DNA region. The screening method of the present invention can be performed using a high throughput method.

In a preferred embodiment of the screening method of the present invention, screening may be performed using the "two-hybrid system" (for example, "MATCHMAKER Two-Hybrid System", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER One-Hybrid System" (all of which are manufactured by Clontech), "HybriZAP Two-Hybrid Vector System" (Stratagene), and methods reported in the literature (Dalton S, and Treisman R (1992) "Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element." Cell 68, 597-612"). More specifically, the method of the present invention may be performed as described below, though it is not to be construed as being limited thereto, and those skilled in the art can appropriately modify the method illustrated below to achieve this invention.

In the two-hybrid system, the TR3 or TINUR protein or a partial peptide thereof is normally fused with the GAL4 DNA binding domain and expressed in yeast cells. Using cells that are predicted to express a protein that binds to the TR3 or TINUR protein or to the partial peptide thereof, a cDNA library is constructed which expresses the protein as a fusion protein fused with a VP16 or GAL4 transcriptional activating region. The library is then introduced into yeast cells, and library-derived cDNAs are isolated from detected

positive clones. (A positive clone can be detected by reporter gene activation caused when a protein that binds to the TR3 or TINUR protein, or their partial peptides including their ligand binding domain, is expressed in yeast cells, and that protein binds to the TR3 or TINUR protein or the partial peptide.) Proteins encoded by the isolated cDNAs can be obtained by transfecting and expressing these cDNAs in *E. coli*. Thus, proteins that bind to the TR3 or TINUR protein or their partial peptide, and genes encoding these proteins may be prepared. Examples of reporter genes that can be used in the two-hybrid system include, but are not limited to, the HIS3 gene, Ade2 gene, LacZ gene, CAT gene, luciferase gene and Plasminogen activator inhibitor type 1 (PAI-1) gene. Screening using the two-hybrid method can also be performed using mammalian cells or the like, in addition to yeast cells.

The present inventors utilized a two-hybrid system that uses mammalian cells, and constructed a high throughput system that can screen for ligands that increase the transcriptional activation function of the TR3 or TINUR protein. This system is an improvement over conventional mammalian two-hybrid systems, and is outlined in Fig. 2 (see Examples below).

In a preferred embodiment, the screening method of this invention is performed using the aforementioned high throughput system, developed by the present inventors.

TR3 or TINUR expression is induced under conditions of leukocyte hyperactivity, as in the peripheral blood during atopic dermatitis. As a result, there is a strong possibility that cell apoptosis will be induced. Ligands that exist *in vivo* can exist in locations where the nuclear receptor is highly expressed. Therefore, the present inventors screened according to the above-mentioned method, using small molecule lipid-soluble mediators predicted to be produced under such conditions as ligand candidate test compounds. Accordingly, the present inventors succeeded in obtaining from among the lipid-soluble mediators the following ligand activators for TR3: prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin A_1,

15-deoxy-delta 12,14-prostaglandin J_2, 8-isoprostaglandin A_1 and such; and for TINUR: prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin J_2, 8-isoprostaglandin A_1 and such. These compounds are prostaglandins comprising a cyclopentenone structure. This shows that ligand activators that up-regulate the transcriptional activating function of TR3 or TINUR can be obtained using a method of this invention.

Screening of compounds that bind to the TR3 or TINUR protein can also be performed using affinity chromatography. For example, the TR3 or TINUR protein can be immobilized on an affinity column carrier, and a test sample predicted to express a protein that binds to the TR3 or TINUR protein is applied thereto. Test samples that can be used in this case include cell extracts and cell lysates. After applying a test sample, the column is washed and any protein that has bound to the TR3 or TINUR protein can be prepared.

A DNA encoding a prepared protein can be obtained by analyzing that protein's amino acid sequence, synthesizing oligo DNAs based on the analyzed sequence, and then screening a cDNA library using those DNAs as a probe.

In the present invention, a biosensor utilizing the phenomenon of surface plasmon resonance may also be used to detect or measure the bound compound. A biosensor utilizing surface plasmon resonance (for example, BIAcore, Pharmacia) uses surface plasmon resonance signals to allow real-time observation of the interaction between the TR3 or TINUR protein and the test compound. Therefore, biosensors such as BIAcore can be used to evaluate binding between the TR3 or TINUR protein and a test compound.

Isolation of compounds that bind to the TR3 or TINUR protein can be routinely performed by those skilled in the art. Methods for screening molecules that bind to a protein of this invention, other than those mentioned above, include methods wherein synthetic compounds, natural product banks or random phage peptide display libraries are acted on the immobilized TR3 or TINUR protein.

A cell used to detect the influence of a candidate compound on

the expression level and transcriptional activation mechanism of the TR3 or TINUR gene, and a polynucleotide or antibody for examining the expression level of this gene, can be combined as a detection kit using a method of the present invention. Candidate compound(s) for use as a positive or negative control, as well as instructions and the like, may be included in the kit. Based on the present invention, a kit for detecting the influence of a candidate compound on the expression level and transcriptional activation mechanism of the TR3 or TINUR gene, may be utilized as a kit for screening compounds that modify the expression level or transcriptional activation mechanism of the TR3 or TINUR gene.

Test candidate compounds that can be used in a screening method of this invention include, without limitation, compound preparations synthesized by chemical methods, such as steroid derivatives; compound preparations synthesized by combinatorial chemistry; mixtures containing multiple compounds, such as extracts from animal or plant tissues, or microbial cultures; purified proteins; expression products of gene libraries; and libraries of synthetic peptides. Furthermore, in a method of screening for compounds that bind to the TR3 or TINUR protein of the present invention, without limitation, it is preferable to use small molecule lipid-soluble mediators as test candidate compounds.

Compounds selected using a method of screening of the present invention are useful as therapeutic agents for allergic diseases. Expression of the TR3 or TINUR gene increases in the eosinophils of atopic dermatitis patients. These apoptosis associated genes may be induced due to negative feedback regulation which acts to reduce the increase in peripheral blood eosinophils that occurs in association with a pathologic condition. Therefore, compounds that can enhance the expression or function of these genes are expected to comprise the action of suppressing the symptoms of atopic dermatitis.

Compounds selected using a screening method of the present invention are expected to serve as allergic disease therapeutic agents that utilize a completely novel functional mechanism that involves TR3 or TINUR activation accompanied by eosinophil apoptosis induction. Therefore, the present invention provides allergic disease

therapeutic agents comprising, as an active ingredient, a compound that can be obtained by a screening method of this invention.

The above-mentioned compound includes compounds in which a portion of the structure of the compound that may be isolated using a screening method of this invention is altered by addition, deletion and/or replacement. As described above, among lipid-soluble mediators, prostaglandins comprising a cyclopentenone structure were found by the present inventors to be compounds that enhance the transcriptional activation ability of TR3 or TINUR (TR3 or TINUR ligand activators). Therefore, examples of allergic disease therapeutic agents according to this invention preferably include those that comprise, as an active ingredient, a prostaglandin that comprises a cyclopentenone structure and that can be obtained using a screening method of this invention. Specific examples of prostaglandins for TR3 include prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin A_1, 15-deoxy-delta 12,14-prostaglandin J_2, 8-isoprostaglandin A_1 and such. Prostaglandins for TINUR include prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin J_2, 8-isoprostaglandin A_1 and such.

Furthermore, substances having TR3 or TINUR receptor ligand activity of the present invention appear to induce eosinophil apoptosis and may have anti-allergic effects. Therefore, the present invention provides apoptosis-inducing agents comprising a TR3 or TINUR receptor ligand as an active ingredient, as well as allergic disease therapeutic agents comprising a TR3 or TINUR receptor ligand as an active ingredient. An apoptosis-inducing agent of the present invention is preferably an eosinophil apoptosis-inducing agent.

Examples of TR3 or TINUR receptor ligands include the above-mentioned prostaglandins comprising a cyclopentenone structure, and the compounds listed in Tables 14 to 49, shown below.

From docking studies of the three-dimensional structure of TR3

and TINUR respectively, one skilled in the art can readily infer, synthesize, and develop synthetic TR3 or TINUR ligands.

Generally, the term "docking study" refers to a computer-mediated search for compounds and conformations that fit into a ligand-binding domain, wherein these compounds and conformations are taken from a 3D database comprising several hundred thousand compounds, and wherein a 3D query pharmacophore model based on the 3D structure of a receptor is used. The docking study can be performed, for example, according to procedures (1) to (4):

(1) Construct a 3D protein structure (homology model) using Modeler;

(2) Search for a binding site using C2.LigandFit;

(3) Construct a pharmacophore query for the binding site using C2.SBF; and

(4) Search a 3D database using the pharmacophore query.

Literature relating to 3D pharmacophore searches includes, for example, Pharmacophore Perception, Development, and Use in Drug Design (Iul Biotechnology Series, 2)-US-ISBN:0963681761 (Hardcover) Guner, Osman F. (Ed.)/Publisher: Intl. Univ. Line Published 1999/12.

Pharmaceutical agents containing such a synthetic ligand as an active ingredient are also included in the allergic disease therapeutic agents of this invention. Furthermore, by using the above-described synthetic ligands as test candidate compounds in an above-mentioned method of this invention, one can evaluate whether or not the synthetic ligand is a true ligand.

Having discovered that expression of the TR3 or TINUR receptor of this invention is specifically induced in eosinophils, the present inventors went on to search for small molecule ligands of these receptors. More specifically, they used a pharmacophore model to simulate the binding site of the PGA derivative of the TR3 ligand-binding region, and based on structure-activity relationship information on the PGA derivative reporter system, they selected from the database compounds other than PGA derivatives matching the binding pocket. Thus, compounds selected as described above are included as ligands of the TR3 or TINUR receptor of this invention. These compounds are shown in Tables 14 to 49. Such compounds may be more useful than agonist antibodies against the receptor of this invention.

The present inventors further discovered that eosinophil CD30 ligand stimulation increases the expression of the TR3 or TINUR gene. Thus, the present invention provides an expression-inducing agent for the TR3 or TINUR gene, which includes a ligand of the eosinophil CD30 receptor. The expression-inducing agent is expected to serve as an allergic disease therapeutic agent that functions by inducing eosinophil apoptosis by regulating expression of genes downstream of TR3 or TINUR in eosinophils.

The therapeutic agents, apoptosis-inducing agents, and gene expression-inducing agents for an allergic disease of this invention can be formulated by mixing an active ingredient with a physiologically acceptable carrier, excipient, diluent or such. The therapeutic agent for an allergic disease of this invention can be administered orally or parenterally, with the aim of improving allergic symptoms.

Oral drugs can be selected from dosage forms such as granules, powders, tablets, capsules, solutions, emulsions, suspensions and so on. Examples of parenteral agents include injections, suppositories and ointments. Injections may include subcutaneous injections, intramuscular injections and intraperitoneal injections.

The dosage of the therapeutic agent for allergic disease according to the present invention may vary depending upon patient age, sex, body weight and symptoms; treatment effects; administration method; treatment duration; and the type of active ingredient contained in the pharmaceutical composition, etc. Generally, the agent can be administered to an adult in the range of 0.1 mg to 500 mg per dose, and preferably 0.5 mg to 20 mg per dose. However, since dose changes with a variety of conditions, a dosage less than that described above may be sufficient in certain cases, and a dosage exceeding this range may be required in others.

The present inventors also discovered that cell apoptosis is induced by increased expression of the TR3 or TINUR receptor protein. Therefore, apoptosis can be induced by activating the TR3 or TINUR protein in cells. Thus, the present invention provides a method of inducing apoptosis of cells that comprises activation of the TR3 or

TINUR receptor protein in these cells. The above method also includes a method wherein cell apoptosis is induced by the activation of TR3 or TINUR gene expression.

In a preferred embodiment of a method of the present invention, apoptosis is induced by contacting cells with a compound, or with a prostaglandin comprising a cyclopentenone structure, where these can be obtained by a screening method of this invention. The cells in a method of this invention are preferably eosinophils. The number of peripheral blood eosinophils is known to decrease in atopic dermatitis patients. Therefore, an allergic disease may be treated by specifically leading eosinophils to cell death, utilizing the method of the present invention. Thus, the present method is expected to lead to the development of novel methods for treating allergic disease.

Since compounds or prostaglandins comprising a cyclopentenone structure obtainable using a screening method of this invention are considered to comprise the function of inducing apoptosis, the present invention also provides apoptosis-inducing agents that comprise these compounds.

The present invention provides a gene whose expression differs in the activated eosinophils of atopic dermatitis patients. The use of the expression of a gene of this invention as an index enables testing for allergic disease and screening for candidate compounds for therapeutic agents.

The expression level of the allergic disease-associated genes of the present invention can be conveniently determined, regardless of allergen type. Therefore, the pathology of allergic reactions can be comprehensively understood.

The method of testing for allergic diseases of the present invention is less invasive for patients because gene expression level can be analyzed using peripheral blood eosinophils as samples. Every year, high throughput and cost effective gene analysis technology methods are being developed. Therefore, it is expected that in the near future, a method of testing for allergic diseases of the present invention will become an important bedside diagnostic tool. Accordingly, a method of the present invention is very valuable from

a diagnostic perspective.

A screening method of the present invention is carried out using, as an index, a genetic function closely associated with eosinophil variation, which is a representative clinical marker for atopic dermatitis. Therefore, compounds that can be found using a screening method of the present invention are expected to be useful for pathological regulation of a wide variety of allergies.

The therapeutic agents for an allergic disease provided by the present invention are also useful as pharmaceutical agents that utilize a completely novel functional mechanism involving TR3 or TINUR activation along with eosinophil apoptosis induction.

Hereinafter the present invention is specifically illustrated with reference to Examples; however, is not to be construed as being limited thereto.

EXAMPLE 1

Differential expression analysis in the peripheral blood eosinophils of atopic dermatitis patients using Affymetrix GeneChip

In order to discover novel therapy-associated genes with fluctuating expression, or genes useful in diagnosis, differential expression comparison analysis using GeneChip was carried out on genes expressed in the peripheral blood eosinophils of healthy subjects, and of atopic dermatitis patients with various pathologic conditions (light and severe steroid sensitivity, severe steroid resistance). This analysis is described below.

Table 2 shows the profiles of six atopic dermatitis patients and two healthy subjects from whom blood samples were drawn. Allergen non-specific (Total IgE), mite-specific and cedar-specific IgEs were measured using the EIA method. More specifically, test sera were allowed to react with an anti-human IgE antibody-bound cap, and allergen non-specific, mite-specific or cedar-specific IgE antibodies in the sera were bound. Next, β-D-galactosidase-labeled anti-human IgE antibody and a substrate solution (4-methylumbelliferyl-β-D-galactopyranoside) were added and reacted, producing a fluorescent substance. The reaction was quenched by adding a quenching solution, and antibody concentration was

determined using the fluorescence intensity of a simultaneously measured standard IgE. L-lactate dehydrogenase (LDH) was measured using the UV method (Wroblewski-La Due method). The rate of NADH decrease caused by its reaction with pyruvic acid was calculated using decreases in absorbance. L-type Wako LDH (Wako Pure Chemicals) and a 7170-type automatic analyzer (Hitachi) were used to measure LDH values. The number of eosinophils was measured using microscopic examination and an automatic hemocyte analyzer SE-9000 (RF/DC impedance system, Sysmex), using 2 ml of EDTA-supplemented blood as the sample.

Table 2

	Healthy subject		Light AD* subject		Severe AD subject (steroid sensitive**)		Severe AD subject (steroid resistant**)	
Sex	Male	Female	Male	Female	Female	Male	Male	Male
Age	23	17	30	25	12	16	24	16
Total IgE (U/ml)	45	25	5	380	**2,400**	**15,000**	**14,000**	**70,000**
Cedar IgE	<0.34	<0.34	<0.34	6.12	<0.34	94.8	60.6	>100
Mite IgE	<0.34	<0.34	<0.34	18.2	**>100**	**>100**	**>100**	**>100**
LDH	228	241	211	296	477	465	303	595

* the dermatitis area <=10% the entire surface area

** sensitivity compared to standard local glucocorticoid therapy.

(1) RNA extraction from peripheral blood eosinophils for use with a DNA chip

A 3% dextran solution was added to whole blood drawn from a patient, and the mixture was left to stand at room temperature for

30 minutes to precipitate erythrocytes. The leukocyte fraction in the upper layer was collected, layered onto Ficoll solution (Ficoll-Paque PLUS; Amersham Pharmacia Biotech), and centrifuged at 1500 rpm for 30 minutes at room temperature. The granulocyte fraction collected in the lower layer was reacted with CD16 antibody magnetic beads at 4°C for 30 minutes. Cells were separated using Magnetic cell sorting (MACS), and cells that eluted without being trapped were used in the experiment as eosinophils.

Eosinophils prepared as described above were dissolved in Isogen (Nippon Gene; Wako Pure Chemicals) and RNA was separated from this solution according to the Isogen protocol. Chloroform was added, the mixture was stirred and centrifuged, and the aqueous layer was collected. Next, isopropanol was added, the mixture was stirred and centrifuged, and the precipitated total RNA was collected. DNase (Nippon Gene; Wako Pure Chemicals) was added to the collected total RNA, the mixture was reacted at 37°C for 15 minutes, and RNA was collected by phenol-chloroform extraction followed by ethanol precipitation. Using these RNAs and a gene chip, analyses were carried out according to the Affymetrix protocol, as detailed below.

(2) cDNA Synthesis for DNA chip

Single stranded cDNA was prepared from 2 μg to 5 μg of total RNA, using reverse transcription with T7-$(dT)_{24}$ (Amersham Pharmacia Biotech) as a primer, Superscript II Reverse Transcriptase (Life Technologies), and the method of the Affymetrix Expression Analysis Technical Manual. The T7-$(dT)_{24}$ primer comprises a nucleotide sequence whereby $d(T)_{24}$ has been added to a T7 promoter nucleotide sequence as shown below.

T7-$(dT)_{24}$ primer:

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-$(dT)_{24}$-3'

(SEQ ID NO: 11)

Next, in accordance with the Expression Analysis Technical Manual, DNA ligase, DNA polymerase I, and RNase H were added to synthesize double-stranded cDNA. The cDNA was extracted by phenol-chloroform extraction, passed through Phase Lock Gels, and then purified using ethanol precipitation.

Biotin-labeled cRNA was synthesized using a BioArray High Yield RNA Transcription Labeling Kit. The cRNA was then purified using an RNeasy Spin column (QIAGEN), and fragmented by heat treatment.

One to 5 μg of this cRNA was added to a hybridization cocktail, in accordance with the Expression Analysis Technical Manual. This was placed into an array and hybridized for 16 hours at 45°C. The DNA chip used was a GeneChip[R] HG-U95A (Affymetrix). GeneChip[R] HG-U95A consists of probes comprising approximately 12,000 kinds of nucleotide sequence, derived from human cDNAs and ESTs.

The DNA chip was washed, and then streptavidin phycoerythrin was added for staining. After washing, a mixed antibody solution of normal goat IgG and biotinylated goat anti-streptavidin IgG antibody was added to the array. To enhance fluorescence intensity, streptavidin phycoerythrin was again added for staining. After washing, the chip was set into a scanner and analyzed using DNA chip analysis software.

(3) DNA chip analysis

Expression fluorescence sensitivity was measured and data analysis was performed using the DNA chip analysis software 'Suite'. First, absolute analysis was performed on all chips, and gene expression level in each of the samples used was measured.

In the analysis of a chip's data, positives and negatives were determined by fluorescence intensity comparison with a probe set of perfect-match and mismatch probes. Using Positive Fraction, Log Avg, and Pos/Neg values, results were evaluated into the three categories of Absolute Call: P (present), A (absent), and M (marginal). Definitions of these terms are shown below:

Positive Fraction: the ratio of positive pairs <u>to probe pairs</u>.

Log Avg: the average of the log of the fluorescence intensity ratio between perfect match and mismatch probe cells.

Pos/Neg: the ratio between the number of positive and negative probe pairs.

Average difference (Avg Diff), which is the average value of the difference in fluorescence intensities between perfect-match and mismatch probe cells, was also calculated.

Genes whose expression varied by more than threefold between patients and healthy subjects were screened, and TR3 was selected from approximately 12,000 HG-U95A chip genes. Since two eosinophil RNA samples from each group, including the healthy groups, were placed onto a gene chip, four combinations of expression comparison, 2x2 between each case, could be carried out. Expression comparison results indicated that TR3 showed a greater than three-fold variation (enhanced in severe cases) in all four combinations between healthy and severe (steroid sensitive) cases (Table 3).

Table 3

Experiment Name	Probe Set	Accession No.	Annotation	Avg Diff	Abs Call	Diff Call	Avg Diff Change	B=A	Fold Change	
C4E307-315	280_g_at	L13740	TR3 orphan receptor	1316	P	I	1208	*	3.7	4(41)
C4E307-340	280_g_at			1234	P	I	1259	*	3.9	
C4E309-315	280_g_at			2042	P	I	1758	*	4.9	
C4E309-340	280_g_at			1913	P	I	1956	*	5.5	

Primers and TaqMan probes used in ABI7700 were designed by Primer Express (PE Biosystems) from sequence information at the National Center for Biotechnology Information (NCBI), and based on accession numbers obtained using Suite. The 5′-end of the TaqMan probe was labeled with FAM (6-carboxy-fluorescein) and the 3′-end was labeled with TAMRA (6-carboxy-N,N,N′,N′-tetramethylrhodamine). The primers and probe used in the TaqMan method are shown below.

Primer 1 (5'): CCACTTTGGGAAGGAAGATGCT (SEQ ID NO: 5)

Primer 2 (3'): ACTTTCGGATGACCTCCAGAGA (SEQ ID NO: 6)

TaqMan probe: ATGTACAGCAGTTCTACGACCTGCTCTCCG (SEQ ID NO: 7)

cDNA prepared from the total RNA by reverse transcription using poly-T (12 to 18 mer) as primers was used as the template. In order to make a standard curve for the calculation of copy number, a plasmid clone containing the nucleotide sequence amplified using both primers was prepared, and serial dilutions thereof were utilized as the template for reaction. The reaction mixture composition for monitoring PCR amplification is shown in Table 4.

Table 4

Reaction mixture composition for ABI-PRISM 7700 (amount per well)

Sterile distilled water	25.66 (μl)
10x TaqMan buffer A	5
25 mM $MgCl_2$	7
dATP (10 mM)	1.2
dCTP (10 mM)	1.2
dGTP (10 mM)	1.2
dUTP (10 mM)	1.2
Forward Primer (100 μM)	0.15
Reverse Primer (100 μM)	0.15
TaqMan Probe (6.7 μM)	1.49
AmpliTaq Gold (5 U/μl)	0.25
AmpErase UNG (1 U/μl)	0.5
Template solution	5
Total volume	50

In order to correct differences in cDNA concentrations among the samples, the same quantitative analysis was carried out for the β-actin gene that was used as the internal standard. The copy number of the target gene was calculated by performing corrections based on the copy number of this β-actin gene. For β-actin gene quantification, human cDNA was used as the template.

The primers and probe accompanying the TaqMan β-actin Control Reagents (PE Biosystems) were used for the measurement of β-actin. Their nucleotide sequences are as follows:

β-Actin forward primer:

TCA CCC ACA CTG TGC CCA TCT ACG A (SEQ ID NO: 12)

β-Actin reverse primer:

CAG CGG AAC CGC TCA TTG CCA ATG G (SEQ ID NO: 13)

β-actin TaqMan probe:

5'-(FAM)ATGCCC-T(TAMRA)-CCCCCATGCCATCCTGCGTp-3' (SEQ ID NO: 14)

FAM: 6-carboxy-fluorescein:

TAMRA: 6-carboxy-N,N,N',N'-tetramethylrhodamine

GeneChip expression analysis is principally aimed at genetic

screening, however as each group had only two samples, the reliability of this screening was confirmed by carrying out an expression comparison between healthy subjects and patients by ABI7700 in a large patient peripheral blood eosinophil panel, in which the number of samples per group was more than ten (Table 5).

Table 5

	No.	Sample ID	Donor ID	Transfer ID	Sex	Age	Toal IgE	Anti-mite IgE	Anti-cedar IgE	LDH	Eosino-phil (%)	Eosinop-hil (mm3)
13 healthy subjects	1	BL10138	V-00026	10138	F	26	5	<0.34	<0.34	105	0	80
	2	BL10140	V-00015	10140	M	52	81	0.71	<0.34	78	2	150
	3	BL10141	V-00040	10141	F	32	59	0.37	<0.34	326	0	40
	4	BL10142	V-00032	10142	F	35	83	14.6	11.2	187	3	250
	5	BL10143	V-00029	10143	F	45	29	<0.34	1.75	113	2	90
	6	BL10144	V-00027	10144	F	29	17	<0.34	1.51	74	2	90
	7	BL10145	V-00034	10145	F	26	120	<0.34	17.1	272	3	590
	8	BL10146	V-00030	10146	F	30	560	<0.34	63.2	251	1	120
	9	BL10147	V-00001	10147	M	50	44	<0.34	17.9	265	4	130
	10	BL10148	V-00003	10148	M	43	220	4	3.54	242	5	250
	11	BL10149	V-00028	10149	M	32	110	1	9.84	245	3	180
	12	BL10150	V-00035	10150	M	63	86	<0.34	12.6	209	5	300
	13	BL10151	V-00019	10151	M	48	42	<0.34	14	300	1	180
15 light cases	14	BL00058	PA00079	9707311	M	0	581				9.7	1390
	15	BL00068	PA00084	9708072	F	13	1687				6.8	365
	16	BL00112	PA00112	9712051	M	2	519				2.2	151
	17	BL00123	PA00120	9712252	F	10	799				12.9	1050
	18	BL00133	PA00129	9712266	M	12	274				1.6	122
	19	BL00198	PA00023	9807213	M	21	9630				15.1	1080
	20	BL00207	PA00181	9807273	F	6	668				8	635
	21	BL00217	PA00190	9808033	M	5	777				22.3	1790
	22	BL00221	PA00042	9808061	F	8	1494				6.6	378
	23	BL00234	PA00029	9808311	F	5	702				6.6	510
	24	BL00252	PA00176	9901071	M	14	2096				7.2	333
	25	BL00259	PA00162	9902161	M	20	2622				13.3	846
	26	BL00270	PA00213	9903292	M	15	230				7.5	368
	27	BL00317	PA00240	0003282	F	14	106	3.77	24.7		2.8	154
	28	BL00327	PA00136	0004033	M	8	1178	<0.35	<0.35		4.4	396
15 moderate cases	29	BL00095	PA00099	9710031	M	3	159				2.5	190
	30	BL00128	PA00124	9712261	M	12	7158				5.2	361
	31	BL00145	PA00048	9802192	F	9	2349				5.1	193
	32	BL00268	PA00179	9903261	M	9	512				9.5	906
	33	BL00278	PA00217	9904061	M	15	1082				22.1	1110
	34	BL00328	PA00175	0004041	M	7	4775	>100	93.3		7.1	638
	35	BL00089	PA00098	9709092	M	7	359				13.3	638
	36	BL00110	PA00110	9711281	F	3	11.5				6.1	198
	37	BL00122	PA00119	9712251	F	12	528				9.7	643
	38	BL00139	PA00025	9801082	M	18	22614				13.7	1140
	39	BL00156	PA00143	9803264	M	6	2625				5	551
	40	BL00287	PA00221	9906231	M	15	1149				3.7	601
	41	BL00296	PA00059	9908201	M	5	1639				6.8	477
	42	BL00323	PA00244	0003302	M	6	4532	>100	69.1		11	909
	43	BL00335	PA00259	BL18526369	F	14	1581	>100	5.46		15.9	1820
18 severe cases	44	BL00078	PA00090	9708251	F	3	135				3.8	254
	45	BL00084	PA00067	9709021	M	3	2149				9.8	1000
	46	BL00163	PA00148	9803304	M	11	137				3:5	274
	47	BL00168	PA00152	9804033	F	19	2732				5.2	261
	48	BL00180	PA00163	9805151	M	17	14758				13.6	1010
	49	BL00242	PA00001	9810061	M	19	13747				13	1230
	50	BL00243	PA00200	9810221	F	6	10967				5.9	662
	51	BL00247	PA00071	9812211	M	16	11610				13.4	972
	52	BL00260	PA00209	9902162	M	0	136				2.5	277
	53	BL00262	PA00120	9902181	F	10	120				3	109
	54	BL00150	PA00137	9803161	F	8	371				4.9	375
	55	BL00257	PA00208	9902053	M	11	268				7.6	468
	56	BL00293	PA00227	9907221	F	10	18301				13.8	1750
	57	BL00298	PA00229	9909141	M	11	9591	>100	18.2		11.9	940
	58	BL00314	PA00238	0002151	M	19	23726	>100	30		6	376
	59	BL00318	PA00241	0003283	F	7	131	<0.35	<0.35		5.7	330
	60	BL00321	PA00243	0003286	F	4	232	<0.35	<0.35		9.1	856
	61	BL00337	PA00261	0005191	F	29	474	52.5	31.6		12.3	797

TR3 expression in peripheral blood eosinophils was confirmed to be significantly enhanced in multiple comparisons of atopic dermatitis patients compared to healthy subjects, and this was largely independent of case severity (Table 6, Fig. 1).

Table 6

CIE-2 L13740	Blood	β- actin (raw) copy/ ng	L13740(raw) copy/ 5ng	copy/ 1ng	β -correction raw(/ng)/average	L13740 correction raw/beta correction
13 healthy subjects	1	253126	1119	224	1.01130301	221
	2	541166	5637	1127	2.16209434	521
	3	214239	2454	491	0.855938946	573
	4	369621	5176	1035	1.476729393	701
	5	716536	6324	1265	2.862741935	442
	6	169173	6969	1394	0.675887508	2062
	7	601310	11426	2285	2.40238633	951
	8	213062	2097	419	0.851236036	493
	9	371589	1266	253	1.484591854	171
	10	646297	1955	391	2.582119848	151
	11	208737	2183	437	0.833956352	524
	12	212114	13130	2626	0.84744903	3099
	13	379539	1205	241	1.516355526	159
15 light cases	14	508758	4893	979	2.032618527	481
	15	248937	6962	1392	0.994564691	1400
	16	221813	12928	2586	0.886198604	2918
	17	315168	11862	2372	1.259174796	1884
	18	141827	11906	2381	0.566636769	4202
	19	244028	17542	3508	0.974953584	3598
	20	348051	14940	2988	1.390552351	2149
	21	387693	20063	4013	1.548931234	2591
	22	268468	4232	846	1.072599907	789
	23	206673	5843	1169	0.825709955	1415
	24	136652	10968	2194	0.545959033	4018
	25	218963	4619	924	0.874812329	1056
	26	209273	3879	776	0.836097009	928
	27	131977	3296	659	0.52728236	1250
	28	121064	22191	4438	0.483680797	9176
6 moderate cases in the remission stage	29	165901		0	0.662815331	0
	30	134119	12595	2519	0.535841346	4701
	31	86340	4693	939	0.344949082	2721
	32	472440	3797	759	1.887519071	402
	33	170914	24513	4903	0.682845244	7180
	34	367818	1497	299	1.469525949	204
9 moderate cases in the exacerbation stage	35	162258	23698	4740	0.648261218	7311
	36	90969		0	0.363443211	0
	37	246460	24652	4930	0.984671042	5007
	38	146805	12808	2562	0.586522301	4367
	39	179179	10603	2121	0.715863818	2962
	40	138858	4884	977	0.554771366	1761
	41	133317	5210	1042	0.532635051	1956
	42	171308	52561	10512	0.684419966	15359
	43	285295	904	181	1.139827753	159
10 severe cases in the remission stage	44	154902	4994	999	0.618872876	1614
	45	78948	12992	2598	0.315418709	8238
	46	231612	4595	919	0.925346905	993
	47	155564	7337	1467	0.621516584	2361
	48	385848	1428	286	1.541561787	185
	49	264744	437	87	1.05772078	83
	50	144715	35283	7057	0.578174465	12205
	51	205943	7545	1509	0.822795017	1834
	52	155395	5335	1067	0.62084169	1719
	53	151703	21933	4387	0.606092505	7238
8 severe cases in the exacerbation stage	54	397821	2000	400	1.589395971	252
	55	446400	5057	1011	1.783480045	567
	56	280724	895	179	1.121564845	160
	57	161385	6500	1300	0.644775207	2016
	58	134978	11103	2221	0.539271624	4118
	59	24740	19712	3942	0.0988414	39885
	60	241793	37484	7497	0.966023991	7760
	61	93068	21520	4304	0.371831799	11575
	total	15268113				

(4) Statistical analysis

Using the above-mentioned data, parametric and non-parametric multiple comparison tests were carried out. Statistical analyses were carried out using an SAS SYSTEM, Version 4.0 Preclinical Package (SAS Institute Inc.). The results are shown in Table 7. Values were significantly greater in the patient group for all of the multiple comparisons between healthy subjects and light cases, healthy subjects and moderate cases, and healthy subjects and severe cases.

Table 7

		C4E HG-U95A statistical analysis results (β-actin-corrected)							
Name of gene		Parametric multiple comparison				Non-parametric multiple comparison			
		Dunnett	p-value	Tukey	p-value	Dunnett	p-value	Tukey	p-value
L13740	TR3 orphan receptor	AS > Nm	0.0533			AL > Nm	0.0339	AM > Nm	0.0189
						AM > Nm	0.01	AS > Nm	0.0378
						AS > Nm	0.0204		

(Nm=normal subject, AL=Light case of atopic dermatitis, AM=Moderate case of atopic dermatitis, AS=Severe case of atopic dermatitis)

Genes indicative of apoptotic character may be enhanced in the peripheral blood eosinophils of patients with atopic dermatitis conditions due to negative feedback regulation, which acts to reduce the increase in peripheral blood eosinophils that occurs in association with a pathologic condition.

EXAMPLE 2

TR3 receptor ligand search

Enhanced TR3 function can be used to promote a pathway that specifically leads eosinophils to cell death. It is highly possible that this will lead to therapies for not only asthma, but also for a variety of allergic diseases including atopic dermatitis, which was investigated by the present inventors. Structurally, TR3 is a nuclear receptor; however, it is an orphan receptor and its native ligand and activator are still unknown. If these can be discovered,

TR3 can be directly activated in eosinophil cells to promote apoptosis. Therefore, it was thought that the ligand activators were highly likely to be anti-allergic agents, and a high-throughput system for ligand screening was constructed.

As shown in Fig. 2, a mammalian two hybrid system was slightly modified by inserting the ligand binding domain sequence or full-length TR3 gene (Fig. 3) into pBIND. This was done to facilitate expression of a protein in which the DNA binding domains of TR3 and GAL4 were fused in frame. A plasmid comprising the TR3 ligand binding domain sequence inserted into pBIND, and a luciferase reporter plasmid comprising a GAL4 binding site, were co-transfected into NIH3T3 cells. Luciferase activity was measured automatically. At this time, activity was also measured by adding a retinoic acid X receptor (RXR) α-gene, which is a transcription factor that forms a heterodimer with TR3. By further adding low molecular weight substances to this system, transcriptional enhancement activity can also be used for screening.

TR3 expression is enhanced in activated eosinophils, such as in the peripheral blood of atopic dermatitis patients. Ligands existing *in vivo* may exist in sites where nuclear receptors are highly expressed. Therefore, small molecule lipid-soluble mediators considered to be produced under such conditions were added to the assay system, and evaluated based on their ability to enhance luciferase activity. Of these lipid-soluble mediators, the activity of enhancing the transcription-activating ability of TR3 was found in prostaglandins comprising a cyclopentenone structure, such as prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 13,14-dihydro-15-keto prostaglandin A_2, 15(R)-15-methyl prostaglandin A_2, and delta12-prostaglandin J2 (Fig. 4; Tables 8 to 12). In this manner, the method established by the present inventors paved the way for the high throughput discovery of native and synthetic TR3 ligands. At the same time the present inventors also found that compounds such as prostaglandin A_2, prostaglandin A_1, and similar metabolites have a high probability of being authentic TR3 native ligands.

Table 8

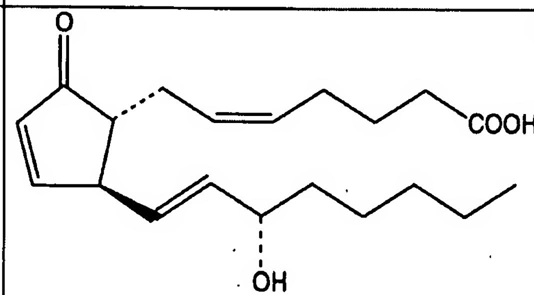
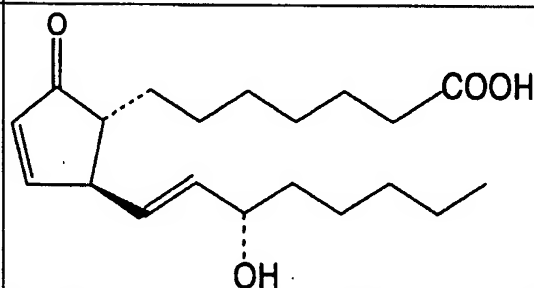
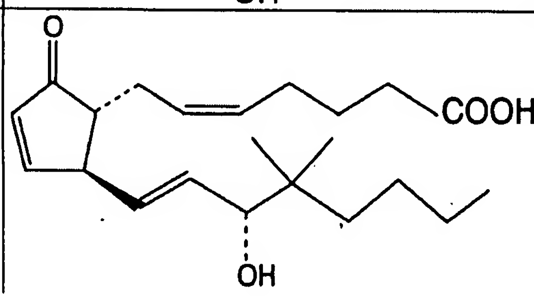
Name of compound	Structural formula	Nur77 LBD-ligand activity		Full length Nur77-ligand activity		Nurr1 LBD-ligand activity		Full length Nurr1-ligand activity	
		RXR(+)	RXR(−)	RXR(+)	RXR(−)	RXR(+)	RXR(−)	RXR(+)	RXR(−)
Prostaglandin A_2		○ 10 μM	×	○ 10 μM	○ 10 μM	○ 10μM	×	○ 10μM	○ 10μM
Prostaglandin A_1		○ 10 μM	×	○ 10 μM	○ 10 μM	○ 10μM	×	○ 10μM	○ 10μM
16,16-dimethyl Prostaglandin A_2		×	×	×	×	×	×	×	×

Table 9

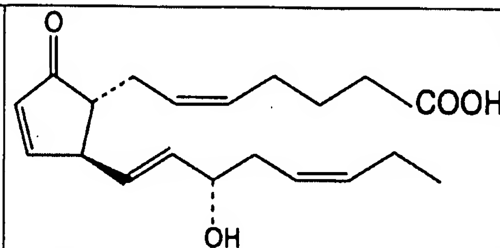
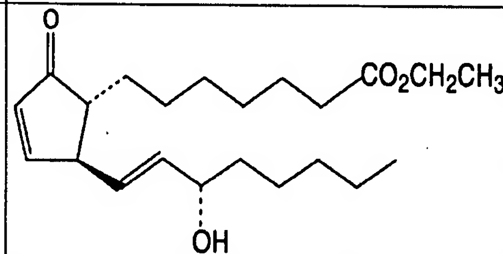
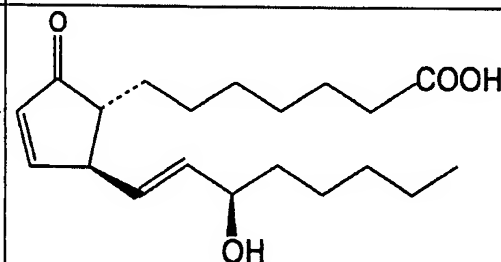
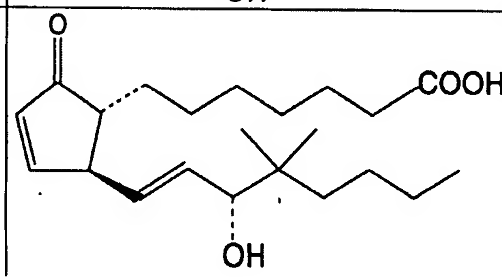
Prostaglandin A_3		×	×	×	×	×	×	×	×
Prostaglandin A_1 ethyl ester		×	×	×	×	×	×	×	×
15-epi Prostaglandin A_1		○ 10 μM	×	○ 10 μM	○ 10 μM	○ 10 μM	×	×	×
16,16-dimethyl Prostaglandin A_1		×	×	×	×	×	×	×	×

Table 10

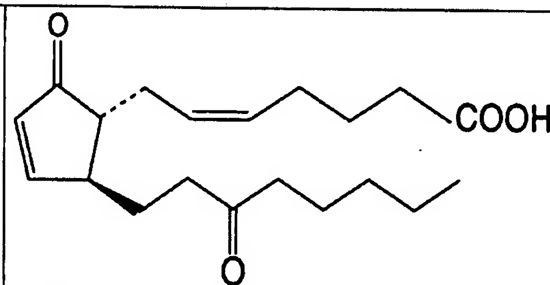
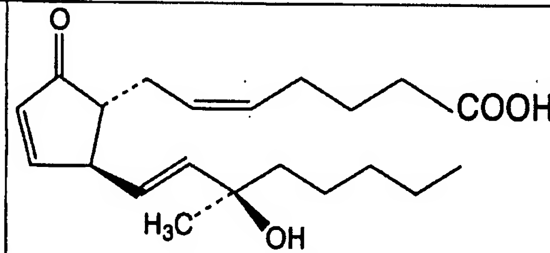
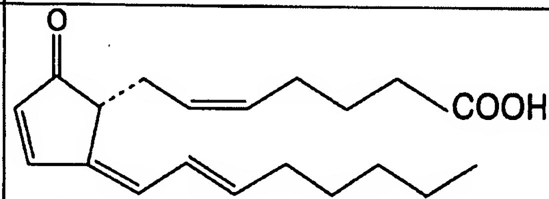
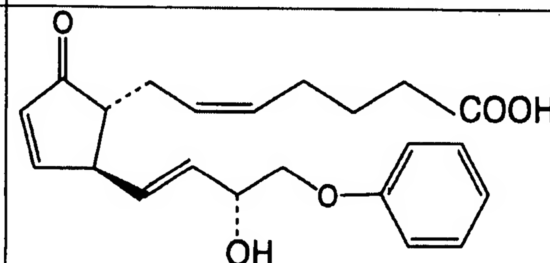
13,14-dihydro -15-keto Prostaglandin A_2		×	×	×	×	×	×	×	×
15(R)-15-methyl Prostaglandin A_2		○ 10 μM	×	○ 10 μM	○ 10 μM	○ 10 μM	×	○ 10 μM	×
15-deoxy-$\Delta^{12,14}$-Prostaglandin A_2		×	×	×	×	×	×	×	×
16-phenoxy tetranor Prostaglandin A_2		×	×	×	○ 30 μM	○ 10 μM	×	○ 30 μM	○ 30 μM

Table 11

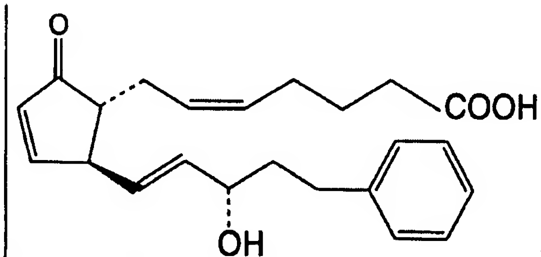
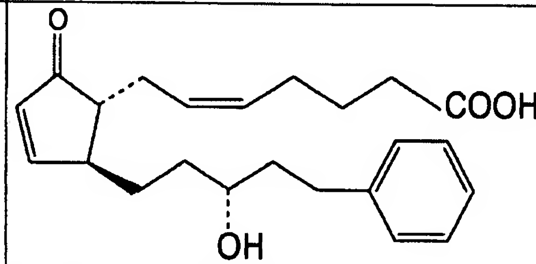
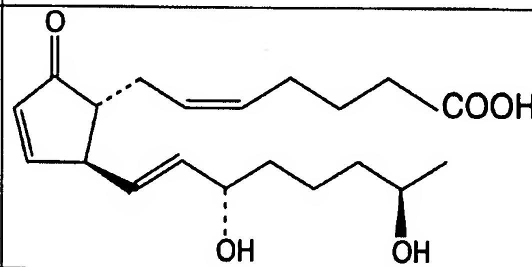
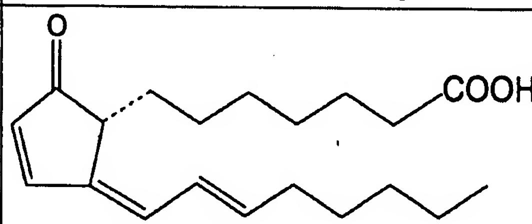
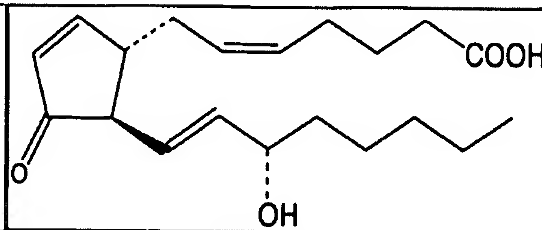
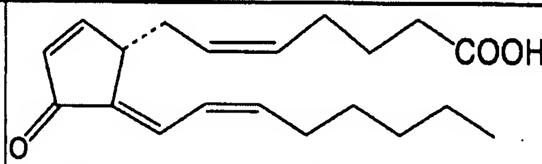
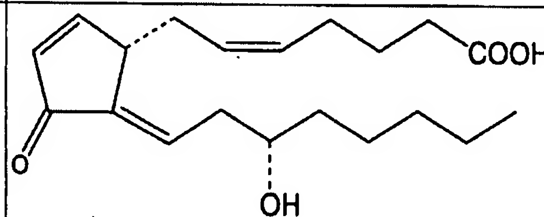
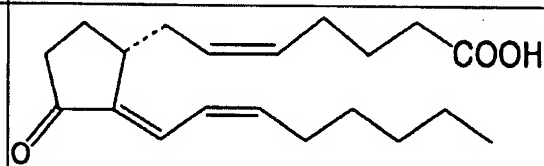
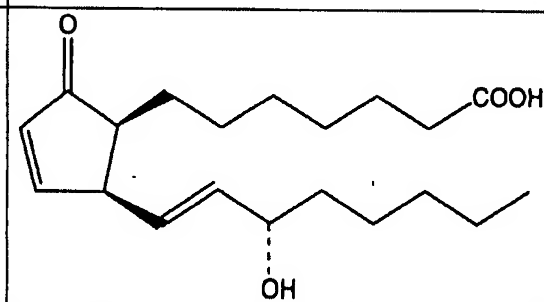
17-phenyl trinor Prostaglandin A_2		○ 10 μM	×	○ 10 μM	×	○ 10 μM	×	○ 10μM	×
17-phenyl trinor-13,14-dihydro Prostaglandin A_2		×	×	×	×	×	×	×	×
19(R)-hydroxy Prostaglandin A_2		×	×	×	×	×	×	×	×
15-deoxy-$\Delta^{12,14}$-Prostaglandin A_1		○ 30 μM	×	○ 30 μM	×	×	×	×	×

Table 12

Prostaglandin J_2		×	×	×	×	×	×	×	×
15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2		×	×	○ 10 μM	○ 10 μM	○ 10 μM	×	×	×
Δ12-Prostaglandin J_2		×	×	×	×	×	×	×	×
9,10-dihydro-15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2 (CAY10410)		×	×	×	×	×	×	×	×
8-iso Prostaglandin A_1		○ 10 μM	ND	○ 3 μM	ND	○ 10 μM	ND	○ 10 μM	ND

EXAMPLE 3

Expression analysis of the TINUR gene

TINUR, a β-type of the nuclear orphan receptor subfamily, was not selected from expression comparison analysis by DD and GeneChip using clinical peripheral blood samples. This receptor's association with specific diseases, including allergic diseases, has not been very clearly elucidated. However, since TINUR was predicted to have functional similarity with TR3, expression comparison with TINUR was carried out between healthy subjects and patients in the same manner as for TR3, that is, using ABI7700 and the same patient peripheral blood eosinophil panel (Table 5) in which the number of examples in a group amounts to more than ten. The primers and probe used for the TaqMan method were the following:

Primer 1 (5'): AGCACAGGCTACGACGTCAA (SEQ ID NO: 8)

Primer 2 (3'): TCTTCTACCTTAATGGAGGACTGC (SEQ ID NO: 9)

TaqMan probe: TTGTACCAAATGCCCCTGTCCGGA (SEQ ID NO: 10)

As shown in Table 13 and Fig. 5, significant enhancement was confirmed in atopic dermatitis patients as compared to normal subjects, regardless of case severity.

Table 13

C1E-2 TINUR	Blood	β– actin (raw)	TINUR(raw)		For β -correction	TINUR-correction
		copy/ ng	copy/ 5 ng	copy/ 1 ng	raw(/ng)/average	raw/beta-correction
13 healthy subjects	1	253126		0	1.01130301	0
	2	541166	81382	16276	2.16209434	7528
	3	214239		0	0.855938946	0
	4	369621	136368	27274	1.476729393	18469
	5	716536		0	2.862741935	0
	6	169173		0	0.675887508	0
	7	601310	203504	40701	2.40238633	16942
	8	213062	78318	15664	0.851236036	18401
	9	371589	121882	24376	1.484591854	16420
	10	646297	105612	21122	2.582119848	8180
	11	208737	165619	33124	0.833956352	39719
	12	212114		0	0.84744903	0
	13	379539	112142	22428	1.516355526	14791
15 light cases	14	508758	146688	29338	2.032618527	14433
	15	248937		0	0.994564691	0
	16	221813	414582	82916	0.886198604	93564
	17	315168	275505	55101	1.259174796	43760
	18	141827	279290	55858	0.566636769	98578
	19	244028	246709	49342	0.974953584	50609
	20	348051	332180	66436	1.390552351	47777
	21	387693	119505	23901	1.548931234	15431
	22	268468	144812	28962	1.072599907	27002
	23	206673	216900	43380	0.825709955	52537
	24	136652	228928	45786	0.545959033	83863
	25	218963	135292	27058	0.874812329	30930
	26	209273	198420	39684	0.836097009	47463
	27	131977		0	0.52728236	0
	28	121064	115898	23180	0.483680797	47923
6 moderate cases in the remission stage	29	165901		0	0.662815331	0
	30	134119	273684	54737	0.535841346	102151
	31	86340		0	0.344949082	0
	32	472440	259151	51830	1.887519071	27459
	33	170914	151666	30333	0.682845244	44422
	34	367818	71428	14286	1.469525949	9721
9 moderate cases in the exacerbation stage	35	162258	519205	103841	0.648261218	160184
	36	90969		0	0.363443211	0
	37	246460	338300	67660	0.984671042	68713
	38	146805	221751	44350	0.586522301	75616
	39	179179	240130	48026	0.715863818	67088
	40	138858	107895	21579	0.554771366	38897
	41	133317	163876	32775	0.532635051	61534
	42	171308	333904	66781	0.684419966	97573
	43	285295	38321	7664	1.139827753	6724
10 severe cases in the remission stage	44	154902	121579	24316	0.618872876	39290
	45	78948	162181	32436	0.315418709	102835
	46	231612	402817	80563	0.925346905	87063
	47	155564	149795	29959	0.621516584	48203
	48	385848	148392	29678	1.541561787	19252
	49	264744	56146	11229	1.05772078	10616
	50	144715	194006	38801	0.578174465	67110
	51	205943	249286	49857	0.822795017	60595
	52	155395	157681	31536	0.62084169	50796
	53	151703		0	0.606092505	0
8 severe cases in the exacerbation stage	54	397821		0	1.589395971	0
	55	446400	263974	52795	1.783480045	29602
	56	280724	54818	10964	1.121564845	9775
	57	161385	102355	20471	0.644775207	31749
	58	134978	85303	17061	0.539271624	31637
	59	24740	44743	8949	0.0988414	90534
	60	241793	322099	64420	0.966023991	66686
	61	93068	135613	27123	0.371831799	72943
	total	15268113				
	Av.	250297				

EXAMPLE 4

TINUR receptor ligand search

Like TR3, TINUR is an orphan nuclear receptor whose native ligands and activators are still unknown. If discovered, they may directly activate TINUR in eosinophil cells and promote apoptosis. Such ligand activators would therefore be anti-allergic agents, and thus a high-throughput system for ligand screening was constructed, using the same methods as for TR3.

The TINUR ligand binding domain sequence or full length gene (Fig. 3) was inserted into pBIND as shown in Fig. 2, in order to facilitate expression of a protein in which the DNA binding domains of TINUR and GAL4 are fused in frame. A plasmid comprising the TINUR ligand binding domain sequence inserted into pBIND, and a luciferase reporter plasmid comprising a GAL4 binding site, were co-transfected into NIH3T3 cells. Luciferase activity was measured automatically. At the same time, activity measurement was also carried out by adding retinoic acid X receptor (RXR) α-gene, a transcription factor that forms a heterodimer with TINUR. Low molecular weight substances can also be added to this system to screen using transcriptional enhancement activity.

Like TR3 expression, TINUR expression is enhanced in activated eosinophils. Ligands existing *in vivo* may exist in sites where nuclear receptors are highly expressed. The native ligands of TR3 were found to be prostaglandin A_2 and prostaglandin A_1. Ligands of the nuclear receptor subfamily are assumed to have structural redundancy. Therefore, derivatives similar to TR3 activator compounds were added, and enhancement of transcriptional activity was investigated. Compounds found to comprise the activity of activating TINUR transcription were prostaglandins comprising a cyclopentenone structure, such as prostaglandin A_2, prostaglandin A_1, 15-epi prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin J_2 and 8-isoprostaglandin A_1 (Fig. 6, Tables 8 to 12). According to the X-ray crystallography results of Wang *et al*, the TINUR (Nurr1) ligand pocket is closed, suggesting it may be a nuclear receptor without a native ligand (Z. Wang, G.

Benoit, J. Liu, S. Prasad, P. Aarnisalo, X. Liu, H. Xu, N. P.C. Walker, T. Perlmann, "Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors" (Tularik Inc.); Nature 423, 29 May, p555-560 (2003)). However, since the above-mentioned reactions are reproducible, and structure-activity relationships exist in compounds similar to prostaglandin A_2, the present inventors revealed that there is a strong possibility that compounds such as prostaglandin A_2, prostaglandin A_1, and similar metabolites are native ligands of not only TR3, but also of TINUR.

EXAMPLE 5

Virtual compounds

A pharmacophore model was used to simulate the binding position of the PGA derivatives to the TR3 ligand binding domain (LBS) (Fig. 7). Based on structure-activity relationship information for the PGA derivative reporter system, compounds other than PGA derivatives that matched the binding pocket were selected from the Catalyst database (screened from BioByte Master File 2001 39,383 compounds, 2,198,646 conformations).

The 158 compounds selected as strongly binding compounds using this simulation are shown in Tables 14 to 45 (including the structural formula). The 117 compounds subsequently selected are shown in Tables 46 to 49.

Table 14

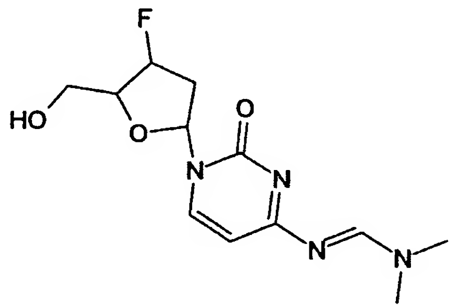
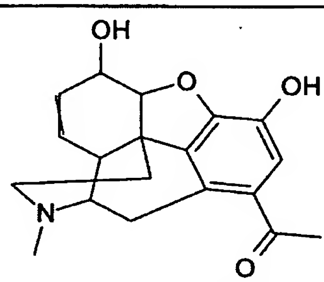
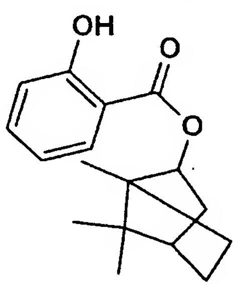
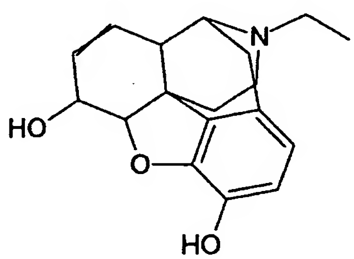
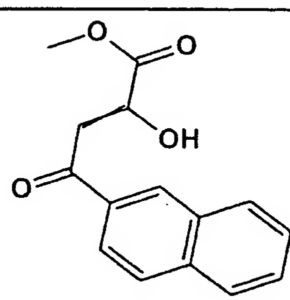
Compound		LUDI_ score	HB_ score	LIPO_ score
	2DEOXY3FLUOROCYTIDINEN4DIMETHYLAMINOMETHYLENE	204	0	325
	1ACETOMORPHINE	158	0	254
	BORNYLSALICYLATE	151	0	272
	NETHYLMORPHINE	136	0	257
	2HYDROXY42NAPHTHALENYL4OXO2BUTENOICACIDMETHYLESTER	126	0	222

Table 15

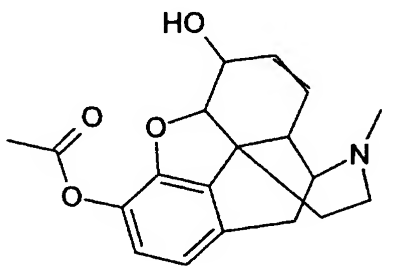
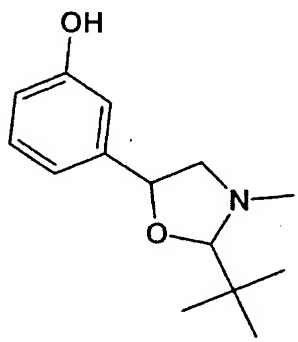
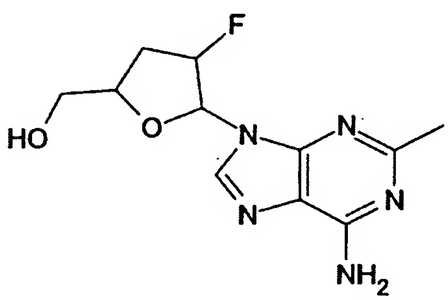
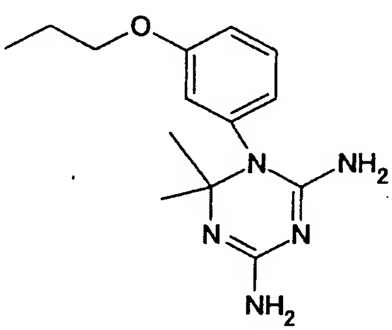
	3ACETYLMORPHINE	123	0	219
	BEREFRINE	112	0	233
	DIDEOXYARAA2METHYL2FLUORO	112	83	150
	STRIAZINE46DIAMINO12H222DIMETHYL13PROPOXYPHENYL	108	0	254

Table 16

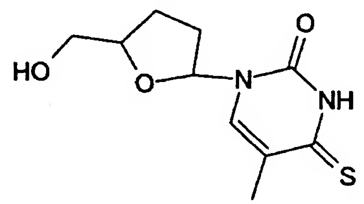
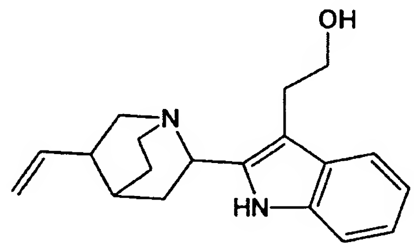
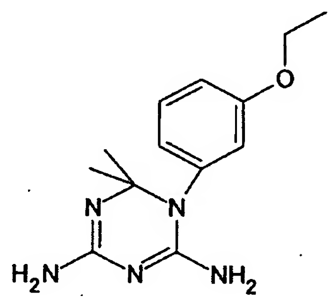
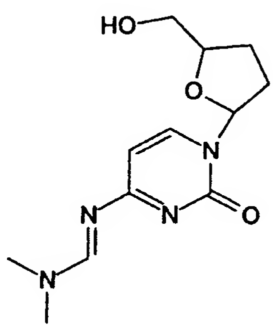
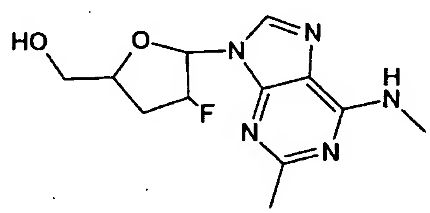
	DIDEOXYTHIOTHYMIDINE	107	83	145
	CINCHONAMINE	103	0	275
	STRIAZINE46DIAMINO12DIHYDRO22DIMETHYL13ETHOXYPHENYL	103	0	224
	23DIDEOXYCYTIDINEN4DIMETHYLAMINOMETHYLENE	101	0	222
	DIDEOXYARAA2N6DIMETHYL2FLUORO	101	0	222

Table 17

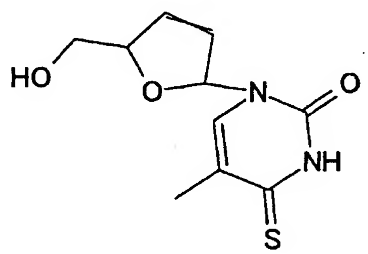
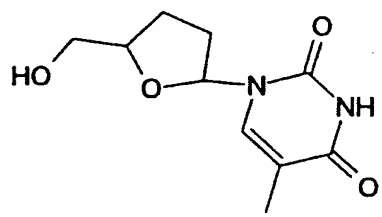
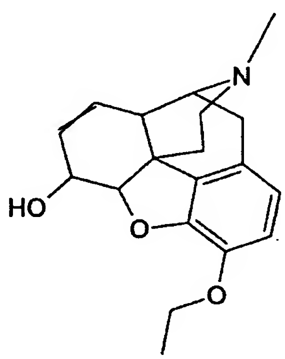
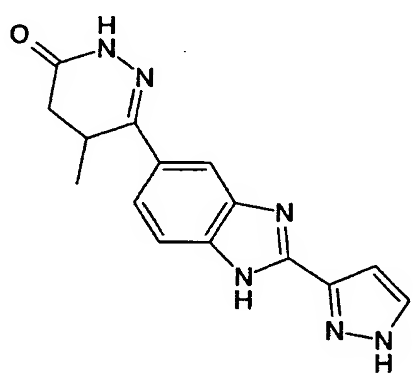
	DIDEOXYTHIOTHYMIDINE23DEHYDRO	101	72	150
	DIDEOXYTHYMIDINE	101	83	139
	ETHYLMORPHINE	101	0	222
	MERIBENDAN	96	0	192

Table 18

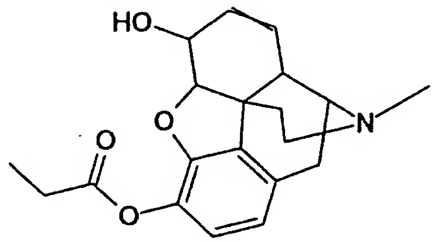
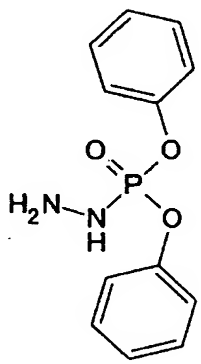
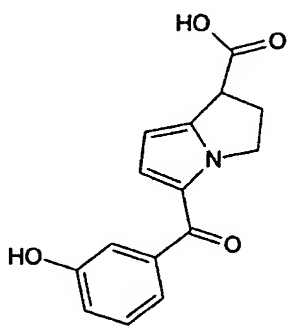
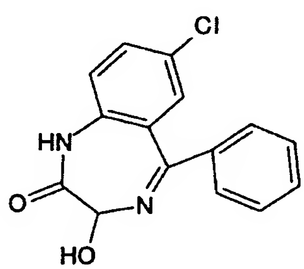
	MORPHINE3PROPIONYL	95	0	216
	PHOSPHOROHYDRAZIDICACIDDIPHENYLESTER	93	0	189
	53HYDROXYBENZOYLH2PYRROLOPYRROLE1CARBOXYLICACID	92	0	213
	OXAZEPAM	90	0	236

Table 19

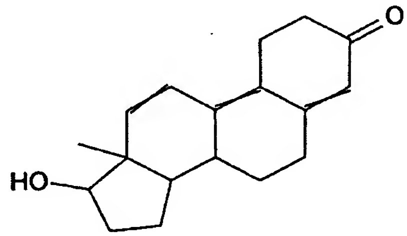
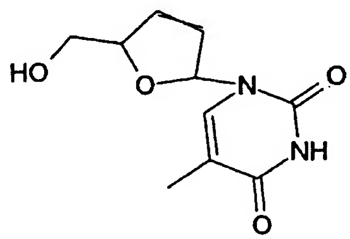
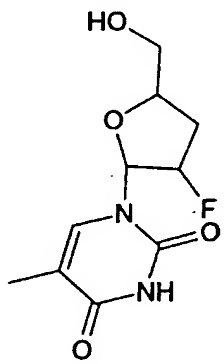
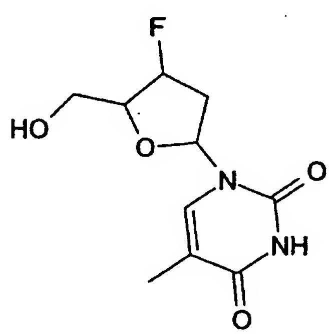
	TRENBOLONE	90	0	186
	STAVUDINE	89	74	136
	THYMINE123DIDEOXY2FLUOROPENTOFURANOSYL	89	83	127
	ALOVUDINE	87	63	145

Table 20

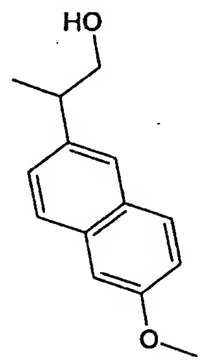
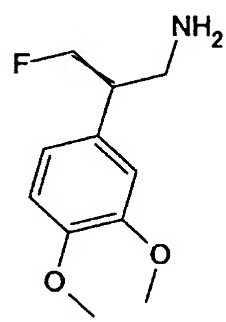
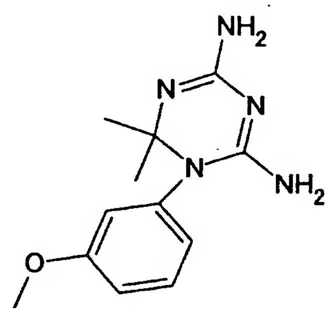
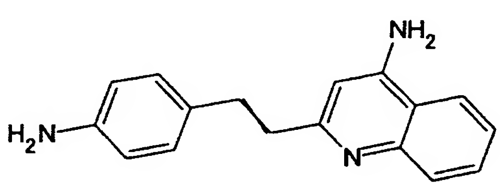
	NAPROXOL	87	0	233
	MDL72638	86	0	207
	12DIHYDROTRIAZINE46DIAMINO22DIMETHYL13METHOXYPHENYL	84	0	180
	4QUINOLINAMINE 2PAMINOSTYRYL	84	0	180

Table 21

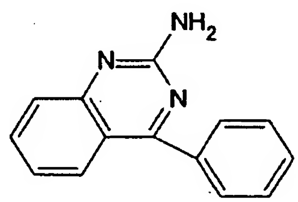
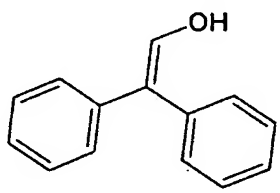
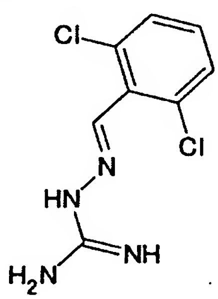
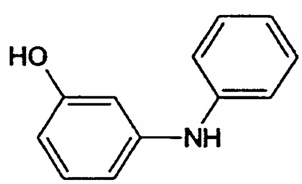
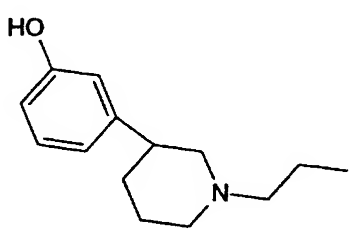
	2AMINO4PHENYL QUINAZOLINE	81	0	177
	DIPHENYLACETAL DEHYDEENOL	81	0	177
	GUANABENZ	81	0	177
	MHYDROXYDIPHE NYLAMINE	81	0	177
	PRECLAMOL	81	0	227

Table 22

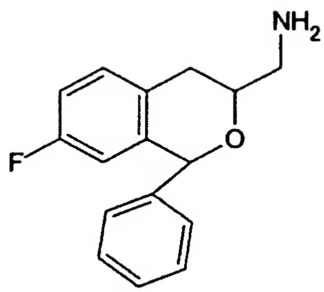
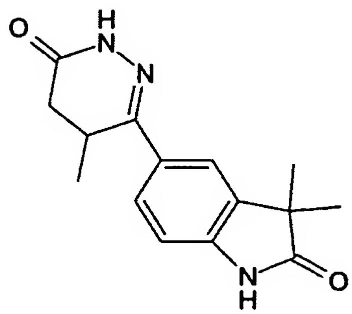
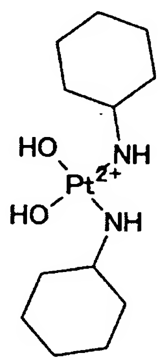
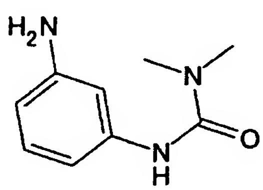
	FENISOREX	77	0	198
	LY195115	75	0	171
	PLATINUMBISCYCLOHEXYLAMMONIODIAQUADINITRATE	75	0	171
	11DIMETHYL33AMINOPHENYLUREA	72	0	168

Table 23

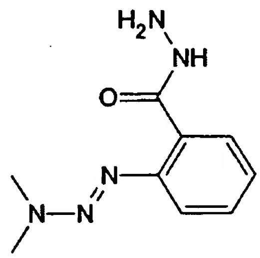
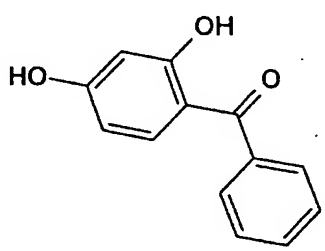
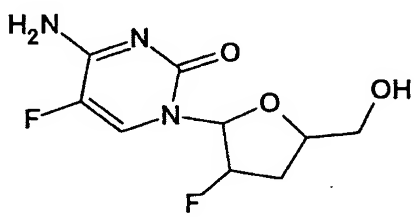
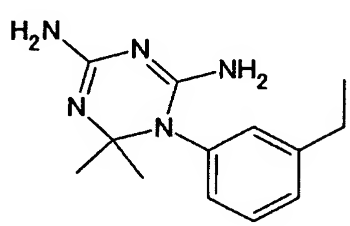
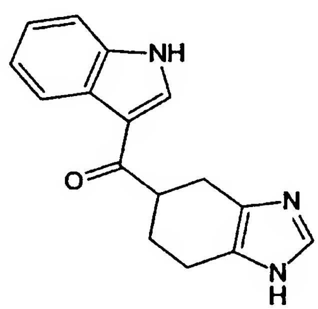
	BENZOICACIDHYDRAZIDEO33DIMETRIAZINO	72	0	168
	BENZOPHENONE24DIHYDROXY	72	0	168
	5FLUOROCYTOSINE123DIDEOXY2FLUOROPENTOFURANOSYL	71	0	192
	STRIAZINE46DIAMINO12DIHYDRO22DIMETHYL13ETHYLPHENYL	71	0	192
	YM060	71	0	192

Table 24

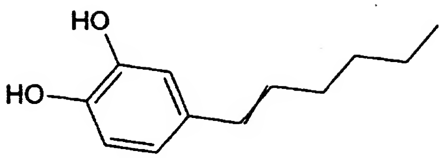
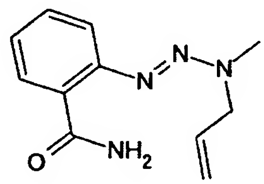
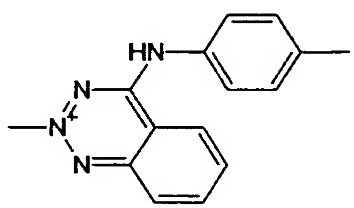
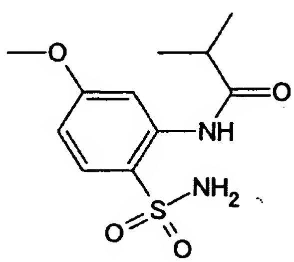
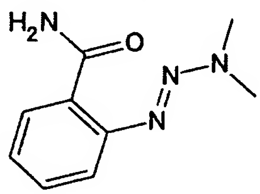
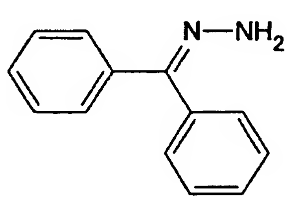
	12DIHYDROXYBENZENE4HEXEN1YL	70	0	242
	BENZAMIDE23METHYL3ALLYLTRIAZENYL	70	0	216
	2METHYL4PTOLYLAMINO123BENZOTRIAZINIUMIODIDE	69	0	165
	BENZENESULFONAMIDE2IBUTYROYLAMINO4METHOXY	67	0	213
	233DIMETHYL1TRIAZINOBENZAMIDE	66	0	162
	BENZOPHENONEHYDRAZONE	66	0	162

Table 25

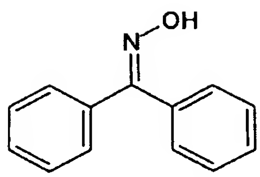
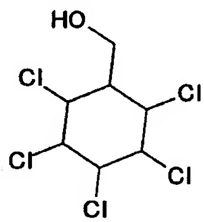
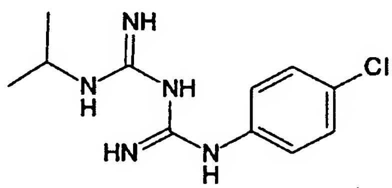
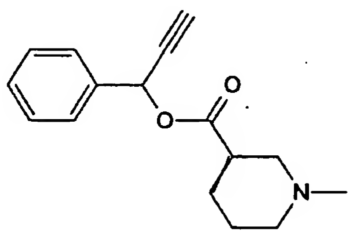
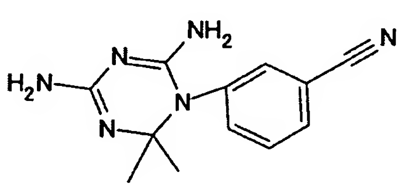
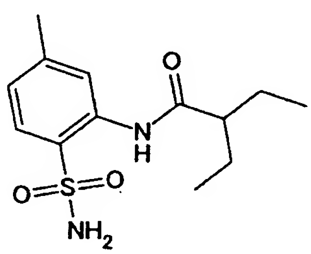
	BENZOPHENONE OXIME	66	0	162
	1HYDROXYMETHYLPENTACHLOROCYCLOHEXANE	65	0	186
	CHLOROGUANIDE	65	0	186
	ARECAIDINEALPHAPHENYLPROPARGYLESTER	64	0	236
	46DIAM22DIME13CYANOPHSTRIAZINE	63	0	159
	BENZENESULFONAMIDE22ETHYLBUTANOYLAMINO4METHYL	63	0	260

Table 26

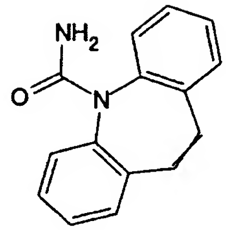
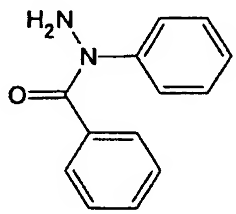
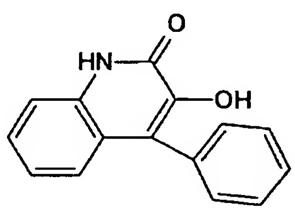
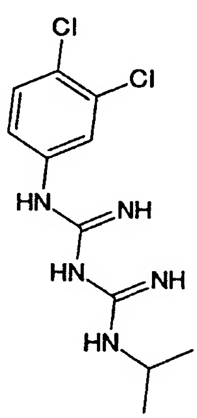
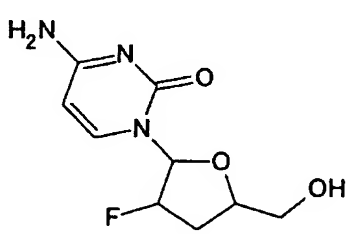
	CARBAMAZEPINE	63	0	159
	N1PHENYLN1BENZOYLHYDRAZINE	63	0	159
	VIRIDICATIN	63	0	159
	CHLORPROGUANIL	62	0	183
	DIDEOXYCYTIDINE2ALPHAFLUORO	62	0	183

Table 27

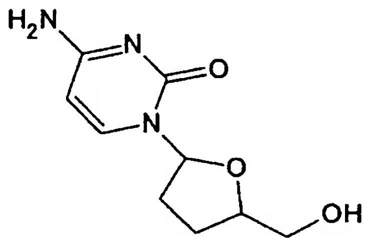
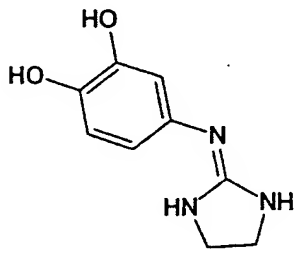
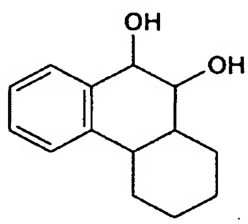
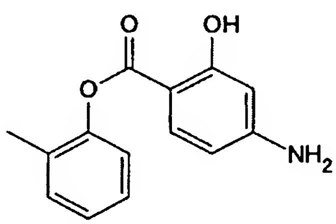
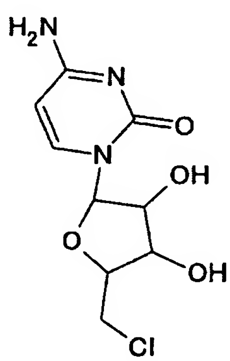
	ZALCITABINE	62	0	183
	234DIHYDROXYPHENYLIMINOIMIDAZOLIDINE	60	0	156
	23BENZOOCTAHYDRONAPHTHALENE34DIOH34DIAX	60	0	156
	4AMINOSALICYLICACID2TOLYLESTER	60	0	156
	5CHLOROCYTIDINE	60	63	118

Table 28

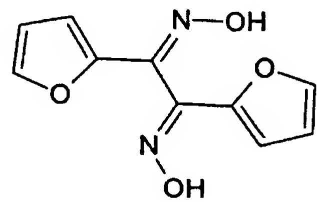
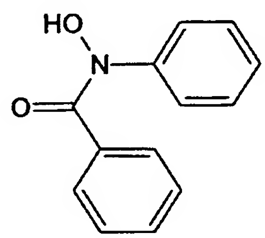
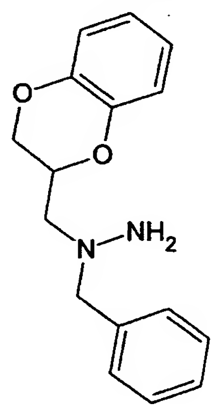
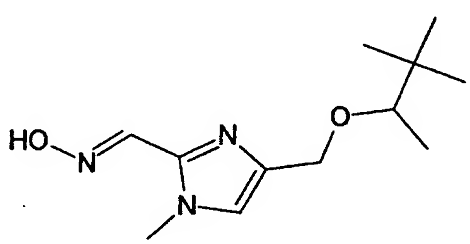
	AFURILDIOXIME	60	0	156
	BENZOYLPHENYL HYDROXYLAMINE	60	0	156
	DOMOXIN	60	0	257
	IMIDAZOLE1METHYL2HYDROXYIMINOMETHYL412DIMETHYLPROPOXYMETHYL	60	0	257

Table 29

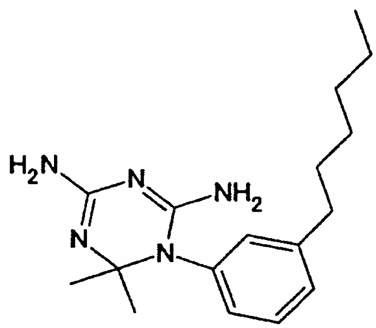
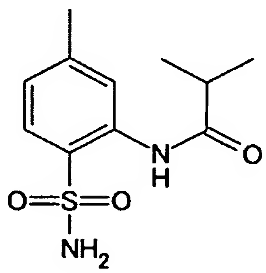
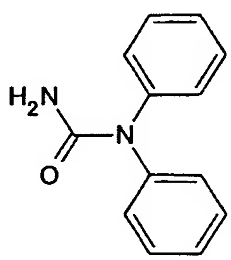
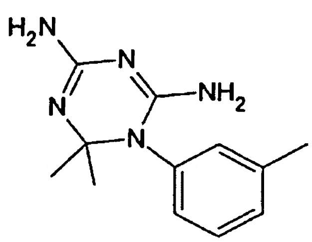
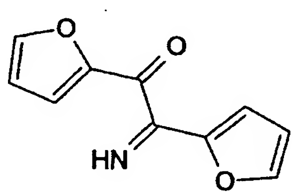
	46DIAM12HSYMTRIAZINE1MHEXYLPHENYL	59	0	281
	BENZENESULFONAMIDE2IBUTYROYLAMINO4METHYL	58	0	204
	11DIPHENYLUREA	57	0	153
	12DIHYDROTRIAZINE22DIMETHYL46DIAMINO13METHYLPHENYL	57	0	153
	AFURILMONOXIME	57	0	153

Table 30

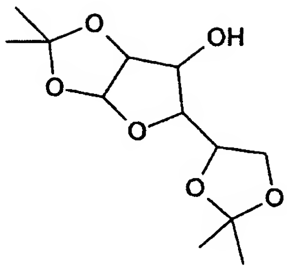
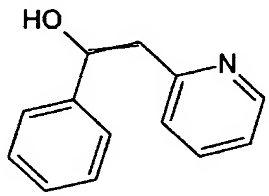
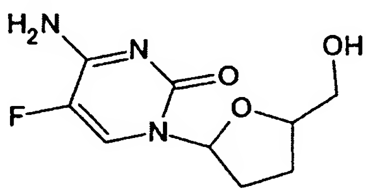
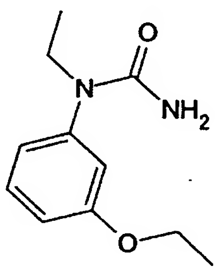
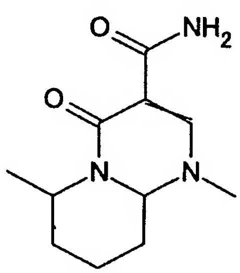
	DIACETONEGLUCOSE	57	0	153
	PYRIDINE2PHENACYLENOL	57	0	153
	DIDEOXYCYTIDINE5FLUORO	56	0	177
	UREA1ETHYL1METHOXYPHENYL	55	0	201
	PYRIDO12APYRIMIDIN4ONE3CONH2H716DIMEAX	54	0	150

Table 31

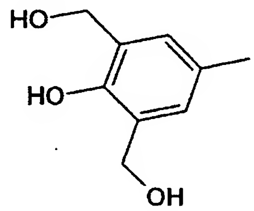
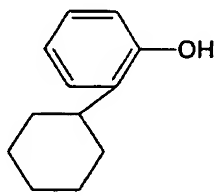
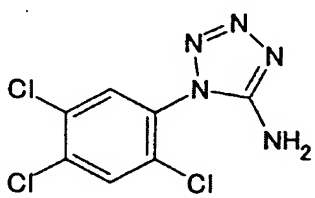
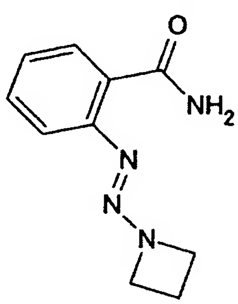
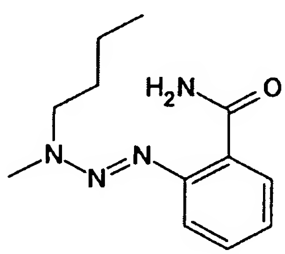
	PHENOL26DIMETHYLOL4METHYL	53	66	133
	2CYCLOHEXYLPHENOL	52	0	148
	5AMINO1245TRICHLOROPHENYLTETRAZOLE	52	0	148
	BENZAMIDE23AZETIDINYLTRIAZENE	52	0	148
	BENZAMIDE23METHYL3BUTYLTRIAZENYL	52	0	224

Table 32

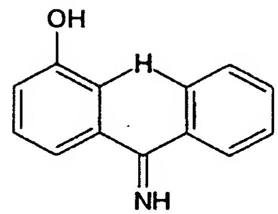
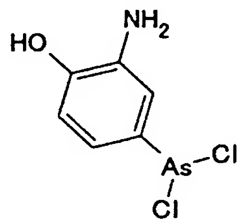
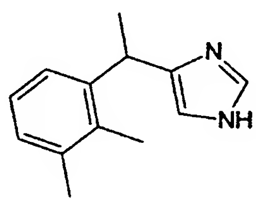
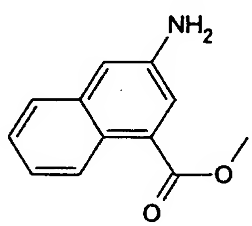
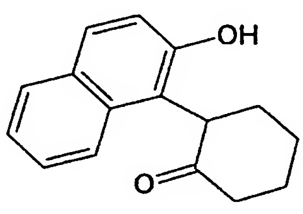
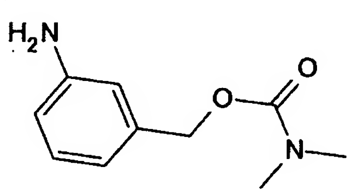
	BENZENEMETHANIMINEA3HYDROXYPHENYL	52	0	148
	DICHLOROPHENARSINE	52	0	148
	MEDETOMIDINE	52	0	198
	NAPHTHALENE2AMINO4METHOXYCARBONYL	52	0	148
	NAPHTHONONE	52	0	148
	NNDIMETHYLCARBAMATEMAMINOBENZYLESTER	52	0	198

Table 33

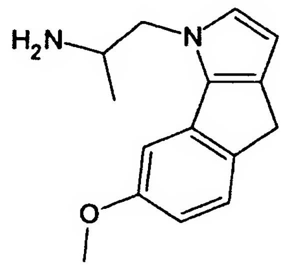
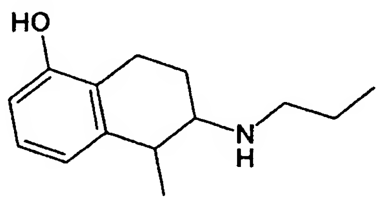
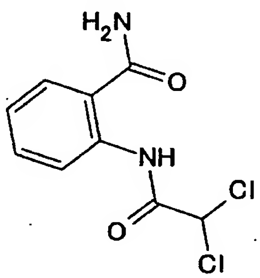
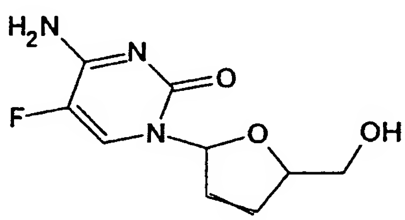
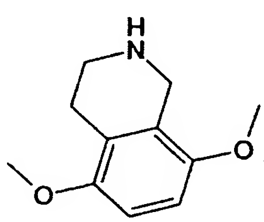
	RO600213	52	0	198
	5HYDROXY1METHYL2AMINOTETRALINNPROPYL	50	0	222
	BENZAMIDEODICHLOROACETYLAMINO	50	0	171
	RA131423	50	0	171
	1234H4ISOQUINOLINE58DIMETHOXY	49	0	145

Table 34

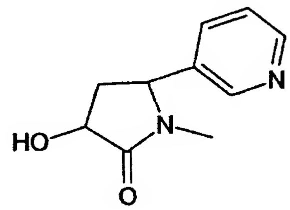
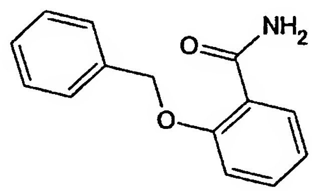
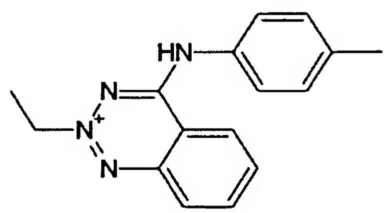
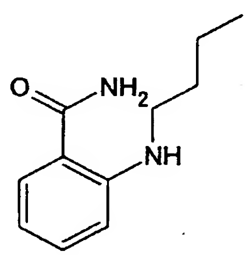
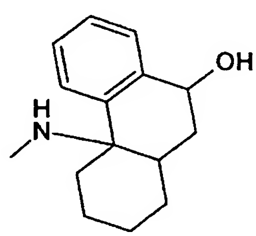
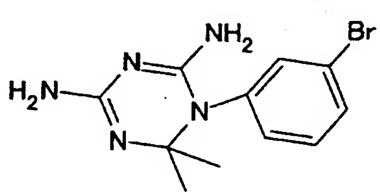
	3HYDROXYCOTININE	49	0	145
	OBENZYLOXYBENZAMIDE	49	0	195
	2ETHYL4PTOLYLAMINO123BENZOTRIAZINIUMIODIDE	47	0	168
	BENZAMIDEOBUTYLAMINO	47	0	219
	OCTAHYDROPHENANTHREN4AAMINENMETHYL9HYDROXY	47	0	168
	13BROMOPHENYL22DIMETHYL46DIAMINOSTRIAZINE	46	0	142

Table 35

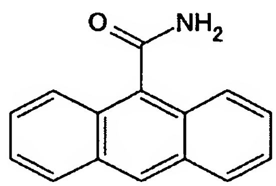
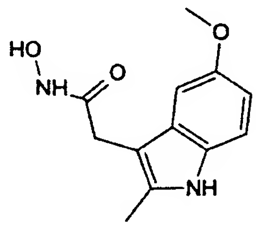
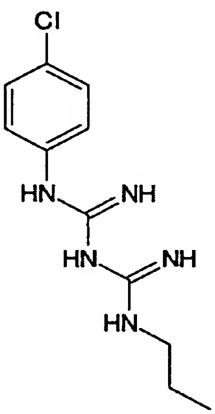
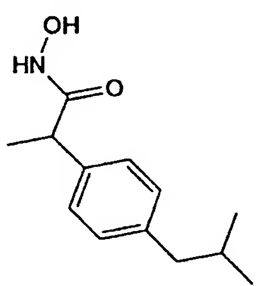
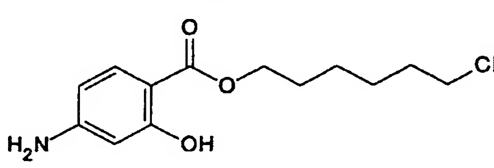
	9ANTHRACENECA RBOXAMIDE	46	0	142
	DEBOXAMET	46	0	192
	N1PCHLOROPHEN YLN5PROPYLBIG UANIDE	46	0	192
	24IBUTYLPHENYL PROPIOHYDROXA MICACID	45	0	242
	PAMINOSALICYLI CACID6CHLOROH EXYLESTER	45	0	293

Table 36

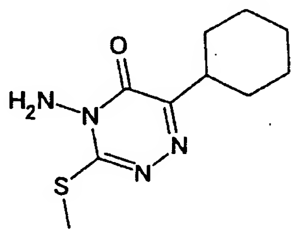
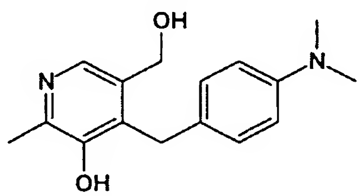
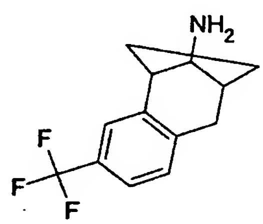
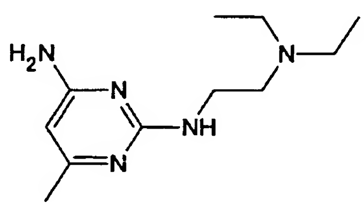
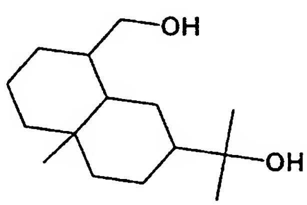
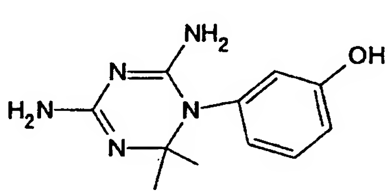
	3METHIO4AMINO6 CYCLOHEXYL124 TRIAZINE5ONE	44	0	165
	4PDIMETHYLANILI NOMETHYLPYRID OXOL	44	0	216
	BENZOCYCLOHEP TANE58METHENO 10AMINO3TRIFLU OROMETHYL	44	0	165
	PYRIMIDINE4AMIN O2DIETHYLAMINO ETHYLAMINO6ME THYL	44	0	266
	TERPENE319717	44	45	145
	12DIHYDROTRIAZI NE22DIMETHYL46 DIAMINO13HYDRO XYPHENYL	43	0	139

Table 37

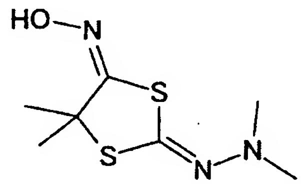
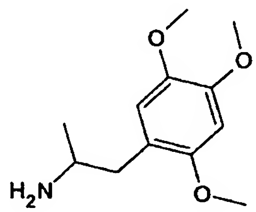
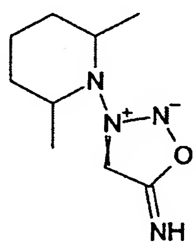
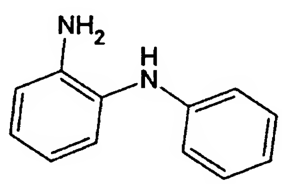
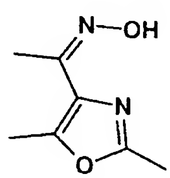
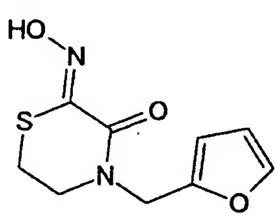
	13DITHIOLANE4OXIMINO2DIMETHYLHYDRAZINO55DIMETHYL	43	0	139
	245TRIMETHOXYAMPHETAMINE	43	0	189
	DARSIDOMINE	43	0	139
	OAMINODIPHENYLAMINE	43	0	139
	OXAZOLE4ACETOXIME25DIMETHYL	43	0	139
	PERHYDROTHIAZIN3ONE2OXIMINON2FURANYLMETHYL	43	0	189

Table 38

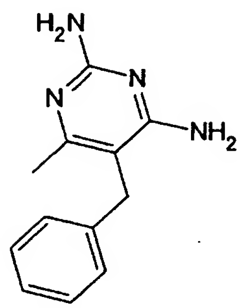
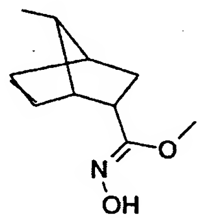
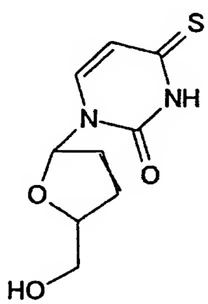
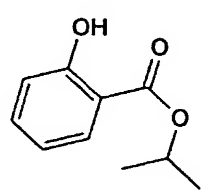
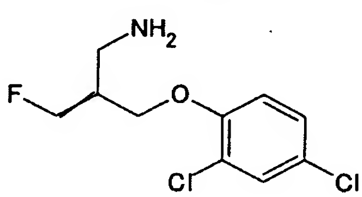
	PYRIMIDINE24DIAMINO5BENZYL6METHYL	43	0	189
	5NORBORNEN2YLHYDROXIMICACIDMETHYLESTER	41	0	162
	DIDEOXYTHIOURIDINE23DEHYDRO	41	0	162
	ISOPROPYLSALICYLATE	41	0	162
	MDL72145	41	0	213

Table 39

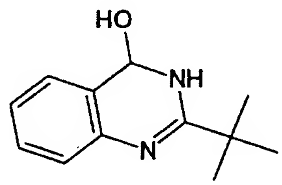
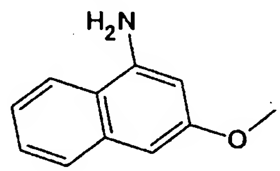
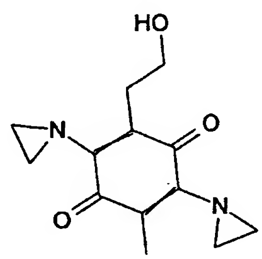
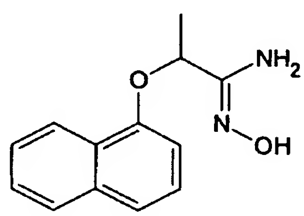
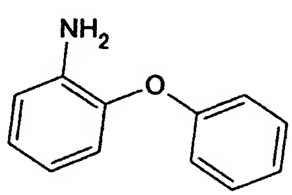
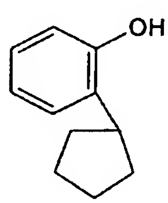
	QUINAZOLINE2TBUTYL34DIHYDRO4HYDROXY	41	0	162
	1NAPHTHALENEAMINE3METHOXY	40	0	136
	BENZOQUINONE25BISAZIRIDINYL3METHYL6HYDROXYETHYL	40	0	186
	NAPRODOXIME	40	0	186
	OPHENOXYANILINE	40	0	136
	PHENOL2CYCLOPENTYL	40	0	136

Table 40

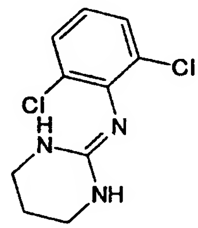
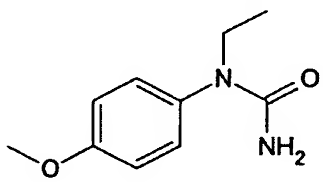
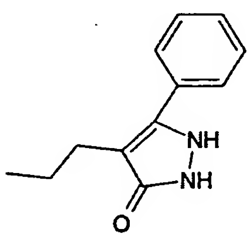
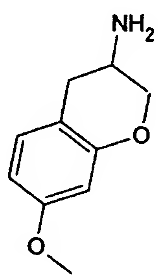
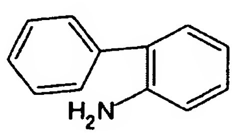
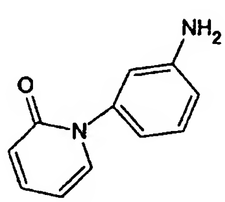
	ST404	40	0	136
	UREA1ETHYL1PA NISYL	38	0	159
	12DIHYDROPYRAZ OLONE4PROPYL5 PHENYL	37	0	183
	24DIMETHOXYAM PHETAMINE	37	0	183
	2AMINOBIPHENYL	37	0	133
	AMPHENIDONE	37	0	133

Table 41

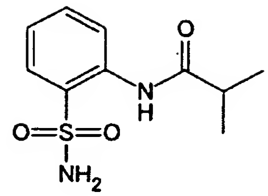
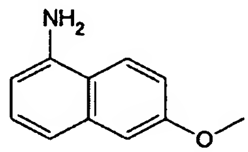
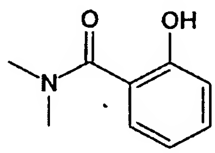
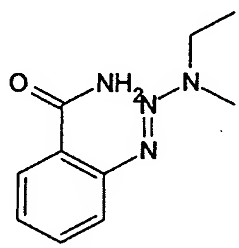
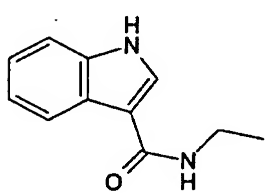
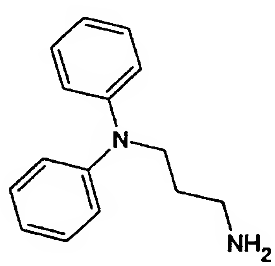
	BENZENESULFONAMIDE2IBUTYROYLAMINO	37	0	183
	NAPHTHALENE1AMINO6METHOXY	37	0	133
	SALICYLAMIDENNDIMETHYL	37	0	133
	BENZAMIDE23METHYL3ETHYLTRIAZENYL	35	0	156
	INDOLE3NETHYLCARBOXAMIDO	35	0	156
	NNDIPHENYLPROPYLENEDIAMINE	35	0	207

Table 42

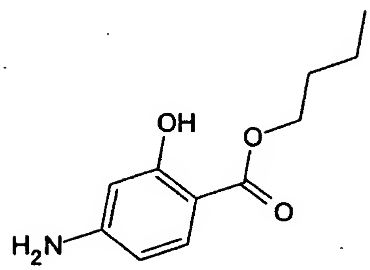
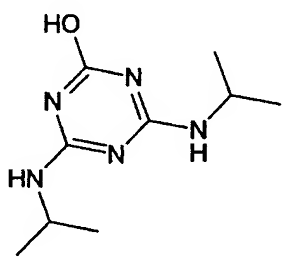
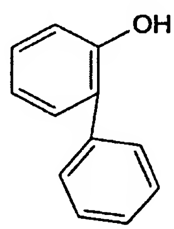
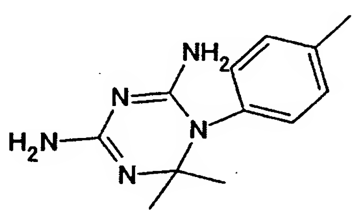
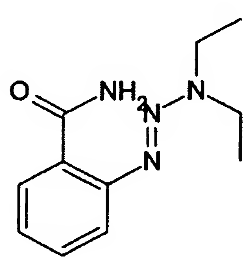
	PAMINOSALICYLICACIDNBUTYLESTER	35	0	207
	2OH46BISIPROPYLAMINOSTRIAZINE	34	0	180
	2PHENYLPHENOL	34	0	130
	46DIAM22DIME14METHYLPHSTRIAZINE	34	0	130
	BENZAMIDE233DIETHYLTRIAZENYL	34	0	180

Table 43

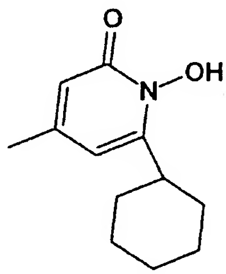
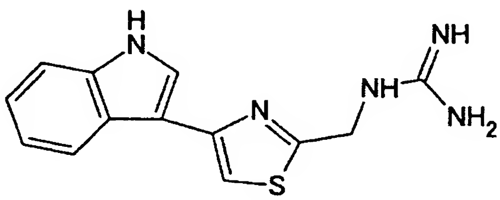
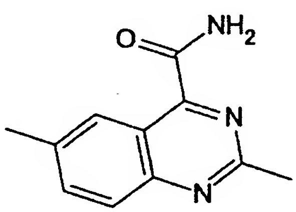
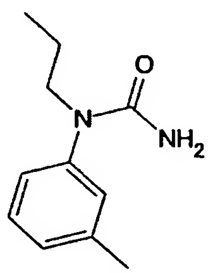
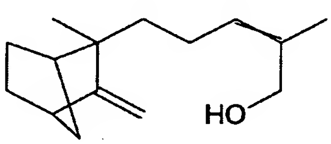
	CICLOPIROX	34	0	130
	INDOLE3THIAZOL 4YL2GUANADYLM ETHYLANALOG	34	0	180
	QUINAZOLINE4CA RBAMOYL26DIME THYL	34	0	130
	UREA1PROPYL1M TOLYL	34	0	180
	BSANTALOL	33	0	230

Table 44

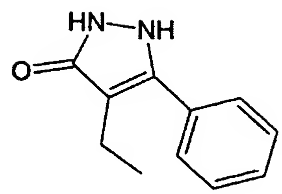
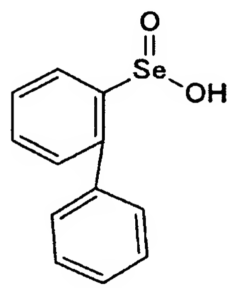
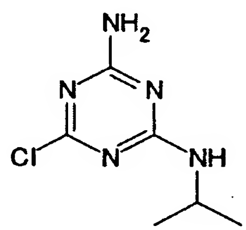
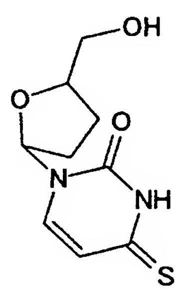
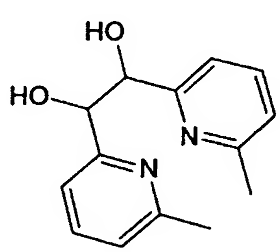
	12DIHYDROPYRAZOLONE4ETHYL5PHENYL	32	0	153
	2BIPHENYLYLSELENIOUSACID	32	0	153
	DESETHYLATRAZINE	32	0	153
	DIDEOXYTHIOURIDINE	32	0	153
	ETHYLENEGLYCOL12BIS6METHYLPYRID2YL	32	0	204

Table 45

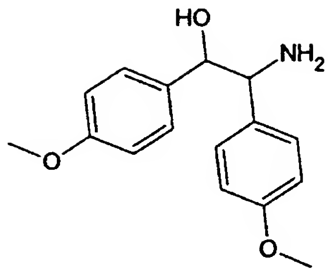
	ISOLADOL	32	0	204

Table 46

Name of compound	MW	LUDI_ score	HB_ score	LIPO_ score	Rule of 5 Violations	Rotlbonds
13HYDROXYPHENYL3METHOXY3METHYLUREA	196.2054	32	0	153	0	5
1HYDROXYPENTACHLOROCYCLOHEXANE	272.3857	28	0	124	0	1
1OHYDROXYMEPHENYL33DIMETRIAZENE	179.2212	18	0	139	0	4
226DIHYDROXYPHENYLIMINOIMIDAZOLINE	193.2048	16	0	112	0	3
24DIAMINO52BR45DIMEOBENZYLPYRIMIDINE	339.1911	19	0	165	0	4
24DIAMINOPYRIMIDINE52CL35DIMEOBENZYL	294.7401	22	0	168	0	4
26DIMETHYL1NAPHTHOL	172.2262	19	0	115	0	1
2ENDOAMINOBENZOBICYCLO222OCTENE	173.2572	25	0	121	0	0
2HPYRAZOLO34AQUINOLIZINE1236710BHEXAHYDRO	177.2486	16	0	112	0	0
2METHOXY4MEAMINO6IPROPYLAMINOSTRIAZINE	197.2394	18	0	139	0	4
2METHYL5IPROPYLPHENOL	150.22	15	0	136	0	2
2OH4ETAMINO6DIETAMINOSTRIAZINE	211.2662	23	0	195	0	6
2OH4IPROPYLAMINO6DIETAMINOSTRIAZINE	225.293	20	0	192	0	6
2PROPYL4PTOLYLAMINO123BENZOTRIAZINIUMIODIDE	279.3639	22	0	168	0	4
2PTERIDINAMINE5678TETRAHYDRO4HYDROXY67DIMETHY	195.2236	16	0	112	0	1
35DIMETHOXYPHENOL	154.1652	16	0	112	0	3
35DITBUTYLPHENOL	206.3272	16	0	162	0	3
3AMINOBENZOICACIDETHYLESTER	165.1914	21	0	142	0	3
3CYCLOHEXENOL1ISOPROPYL4METHYL	154.2516	18	0	139	0	2
3HYDROXY4METHOXYCINNAMICACIDETHYLESTER	222.2402	18	0	139	0	6
3OPENTYLMORPHINE	355.476	16	0	213	0	6
4HYDROXYETHYLVANILLIN	196.2024	31	0	177	0	6
4QUINOLINAMINE2METHYL	158.2024	22	0	118	0	0
4QUINOLINAMINE6ETHOXY24PHENYLBUTADIENYL	316.4018	15	0	136	0	5
5METHOXY8QUINOLINOL	175.1866	28	0	124	0	2
6METHYL5INDANOL	148.2042	16	0	112	0	1
8QUINOLINAMINE6METHOXY	174.2018	25	0	121	0	1
AAMIDOETHYLCINNAMATE	219.2396	27	0	148	0	5
AAMIDOMETHYLCINNAMATE	205.2128	28	0	124	0	4
ANILINE35DIMETHOXY	153.1804	22	0	118	0	2
ANILINE35DITBUTYL	205.3424	19	0	165	0	2
ANTHRALIN102HYDROXYETHIO	302.3442	17	0	189	0	6

Table 47

ATROMEPINE	303.4004	22	0	219	0	6
BENZAMIDENHEXYL34DIHYDROXY	237.298	29	0	251	0	9
BENZAMIDEOISOPROPYLAMINO	178.2334	15	0	136	0	3
BENZENEMETHANIMINE25DIMETHYLAPHENYL	209.2902	16	0	112	0	2
BENZENESULFONAMIDE22ETHYLBUTANOYLAMINO	270.3458	22	0	219	0	6
BENZOICACID2AMINOMETHYLESTER	151.1646	19	0	115	0	2
BENZOICACIDHYDRAZIDEO33DIMETRIAZINO	207.2346	40	0	136	0	4
BENZOIN	212.2476	16	0	162	0	4
BENZOINOXIME	227.2622	28	0	174	0	5
BENZYLALCOHOL35DIMETHOXY4HYDROXY	184.1914	21	0	142	0	5
CARVEOL	152.2358	18	0	139	0	2
CINAMETICACID	238.2396	16	0	162	0	8
CYPENAMINE	161.2462	25	0	121	0	1
CYTIDINE23DIDEHYDRO23DIDEOXY	209.2042	15	0	136	0	3
CYTIDINEDIDEOXY3FLUORO	229.2105	18	0	139	0	3
CYTOSINE2BUTOXY	167.2102	17	0	189	0	4
DMDC	239.2304	27	0	148	0	4
ECGONINEMETHYLESTER	199.2492	27	0	148	0	3
ETHYCHLOZATE	238.6731	20	0	192	0	4
ETHYLENEGLYCOLMONO24DICHLOROPHENYLETHER	207.056	25	0	171	0	4
ETHYLMETHYLGLYOXIME	130.1462	27	83	65	0	4
F11105	203.2432	15	0	136	0	2
FLOVERINE	198.2182	22	0	168	0	6
GUANIDINE1METHYL14CHLOROPHENYL	183.6401	19	0	115	0	2
GUANIDINEN43AMINOPHENYLTHIAZOL2YL	233.2904	22	0	118	1	2
HEXAHYDROFLUOREN9AAMINE	187.284	28	0	124	0	0
ILEPRO	228.2906	16	83	130	0	6
IMIDAZOLINE22HYDROXYPHENYL	162.1908	19	0	115	0	2
INDOLE3CARBOXYLICACIDETHYLESTER	189.2134	18	0	139	0	3
INDOLE3IMIDAZOL1YLMETHYL	197.239	16	0	162	0	2
INDOLE3NMETHYLCARBOXAMIDO	174.2018	28	0	124	0	2
LAMIVUDINE	229.2532	18	0	139	0	3
METHYLBENZOATE2AMINO5CHLORO	185.6097	16	0	112	0	2
METHYLSALICYLATE	152.1494	16	0	112	0	3

Table 48

MORPHINE3HEXANOYL	383.4864	25	0	222	0	7
MPENTOXYPHENOL	180.2462	16	0	213	0	6
N1PCHLOROPHENYLN5METHYLBIGUANIDE	225.6803	43	0	139	0	5
N2N4N6TRIMETHYLNNNHYDROXYMETHYLMELAMINE	258.2796	17	3	186	0	9
NAPHTHALENE1AMINO3CHLORO	177.6329	25	0	121	0	0
NAPHTHALENE1AMINO3METHYL	157.2146	25	0	121	0	0
NAPHTHALENE1AMINO6CHLORO	177.6329	28	0	124	0	0
NBUTYLSALICYLIDENEIMINE	177.2456	20	0	192	0	5
NCYCLOPENTYLCINNAMAMIDE	215.2944	27	0	148	0	4
NETHYLMORPHINE	299.3688	136	0	257	0	3
NHYDROXYETHYLPTP	203.2834	31	0	177	0	4
NITRAFUDAM	231.2104	25	0	121	0	2
NNDIMETHYLTRYPTAMINE6METHOXY	218.298	29	0	201	0	4
OMETHOXYBENZAMIDE	151.1646	16	0	112	0	2
OMETHYLCINNAMAMIDE	161.203	22	0	118	0	2
OMETHYLTYROSINEETHYLESTER	223.2712	30	0	227	0	6
PAMINOSALICYLICACID4CHLOROBUTYLESTER	243.6895	22	0	219	0	7
PAMINOSALICYLICACIDNAMYLESTER	223.2712	25	0	222	0	7
PENTA24DIENYLAMINE23455PENTACHLORO	255.3583	18	0	139	0	2
PENTALAMIDE	207.2718	19	0	216	0	6
PHENOL2HEPTYL	192.3004	21	0	142	0	7
PHENYLBORONICACIDMETHOXYACETAMIDO	209.0081	19	0	165	0	7
PICOLINHYDROXAMICACID	166.1792	19	0	115	0	3
PROTOCATECHUICACIDETHYLESTER	182.1756	15	0	136	0	5
PYRAZINE2AMIDINO56DIMETHYL3METHYLAMINO	179.2242	25	0	121	0	2
PYRAZOLE23DIHYDRO3IMINO15DIMETHYL2PHENYL	187.2438	25	0	121	0	1
PYRAZOLE24DIMETHYL5PHENYL	172.2292	16	0	112	0	1
PYRAZOLE426DIMETHYLPHENYLMETHYL	186.256	16	0	162	0	2
PYRAZOLE4METHYL5PHENYL	158.2024	16	0	112	0	1
PYRIDINE22HYDROXYPHENYL	171.1982	25	0	121	0	2
PYRIDINE4HYDROXY26BISMETHOXYCARBONYL	211.1738	22	0	118	0	5
PYRIMIDINE24DIAMIO6METHYL5PHENYL	200.2426	22	0	118	0	1
PYRIMIDINE2AMINO4DIETHYLAMINOETHYLAMINO56DIMETHYL	237.3472	26	0	248	0	6

Table 49

PYRIMIDINE2DIMETHYLAMINO4METHYLAMINO	152.1986	19	0	115	0	2
PYRIMIDINE2HYDRAZINO4METHOXY6METHYL	154.1712	19	0	115	0	2
PYRIMIDINE4AMINO2DIMETHYLAMINO	138.1718	28	0	124	0	1
QUINOLINE4AMINO7CHLORO	178.6207	22	0	118	0	0
RA161045	371.484	19	0	216	0	5
SYMTRIAZINE2ETHYLAMINO4TBUTYLAMINO6HYDROXY	211.2662	22	0	168	0	5
TERPENE319712	268.3954	25	0	171	0	4
TETRAHYDROPYRAN24DIONE31ETHOXYIMINOBUTYL66SPIRO35DIMETHYLCYCLOHEXYL	323.4314	23	0	195	0	6
TIMIRDINE	227.7111	16	0	112	0	1
TIZOLEMIDE	335.8229	27	0	148	0	3
UREA1BUTYL1PTOLYL	206.287	17	0	189	0	5
UREA1ETHYL1MTOLYL	178.2334	29	0	150	0	3
UREA1ETHYL1OANISYL	194.2328	29	0	150	0	4
UREA1ETHYL1OETHOXYPHENYL	208.2596	31	0	177	0	5
UREA1METHYL1MTOLYL	164.2066	22	0	118	0	2
VERBENOL	152.2358	19	0	115	0	1
VESTITOL	272.3	22	0	118	0	4

EXAMPLE 6

Decrease of activity by LBD deletion mutant

Prostaglandin A_2 transcriptional activity was suppressed in a Mammalian Two Hybrid reporter system that used a TR3 or TINUR gene completely lacking an LBD region (Fig. 8). Thus, it was implied that prostaglandin A_2 functions by acting on the LBD region of the nuclear receptor.

EXAMPLE 7

Demonstration of the binding of PGA derivatives to TR3 or TINUR using BIAcor

To conclusively demonstrate PGA derivative ligand binding activity to TR3 or TINUR, revealed using the Mammalian Two Hybrid reporter system, TR3 GST-LBD and TINUR GST-LBD were respectively expressed in *E. coli*, and then purified. PGA1 and PGA2 binding to the LBD of TR3 or TINUR was detected by BIAcor S51, using comparison with GST as a base (Fig. 9). The negative control compound, 13,14-dihydro-15-keto-PGA2, did not demonstrate any activity in the reporter system, and did not bind to the LBD.

EXAMPLE 8

Genes such as TR3 or TINUR, which comprise apoptotic character, may be enhanced in the peripheral blood eosinophils of atopic dermatitis conditions due to negative feedback regulation that acts to reduce the increase in peripheral blood eosinophils that occurs in association with a pathologic condition. Therefore, the present inventers investigated *in vitro* the type of stimulation that causes expression of this kind of gene in eosinophils.

A large number of peripheral blood eosinophils were collected from healthy subjects and cultured, while suppressing their activation, in suspension in petri dishes. Eosinophil activation by stimulation with cytokines such as IL-5 and IL-4 did not lead to TR3 induction. In contrast, induction of cell apoptosis using anti-CD30 antibody resulted in dramatic induction of TR3 and TINUR in cultured peripheral blood eosinophils over a one to three-hour treatment (Table

50, Figs. 10 and 11). This anti-CD30 antibody comprises agonist activity towards eosinophil CD30, and has recently received attention due to possible use as a therapeutic agent for asthma or the like, by inducing apoptosis in eosinophils by a specific intracellular pathway. Table 50 below summarizes the apoptosis induction of human peripheral blood eosinophils.

Table 50

		Annexin V-positive cells (%)
Fresh		4.0
Control	1 hr	2.30
Anti-CD30 antibody		9.20
Anti-Fas antibody		5.20
Control	3 hr	4.50
Anti-CD30 antibody		20.00
Anti-Fas antibody		13.80
Control	24 hr	11.70
Anti-CD30 antibody		63.00
Anti-Fas antibody		31.20

Although the anti-Fas antibody induced apoptosis, albeit more slowly than the anti-CD30 antibody, it did not induce TR3 and TINUR. Thus, apoptosis induction by the anti-CD30 antibody, accompanied by TR3 and TINUR induction, may occur through an eosinophil-specific apoptosis pathway that is different from conventional pathways. These phenomena (apoptosis induction and expression induction of TR3 or TINUR) were similarly observed when AML14.3D10, an eosinophil-specific cell line, was treated with anti-CD30 antibody (Figs. 12, 13 and 14).

It is very likely that promotion of a pathway that specifically leads eosinophils to cell death through the enhancement of TR3 or TINUR function will lead to the treatment of not only asthma, but also of various allergic diseases including atopic dermatitis, which was investigated by the present inventors. An example of the

therapeutic strategy intended by the present inventors is shown in Fig. 15.